



**EUROPEAN COMMISSION**  
Directorate-General for Research and Innovation  
Prosperity  
**Sustainable Industry Systems**



## **GRANT AGREEMENT**

**NUMBER 870292 — BioICEP**

This **Agreement** ('the Agreement') is **between** the following parties:

**on the one part,**

the **European Union** ('the EU'), represented by the European Commission ('the Commission'),

represented for the purposes of signature of this Agreement by Head of Unit, Directorate-General for Research and Innovation, Innovative Administration, Financial Management and Program Support III, Jacques VAN OOST,

**and**

**on the other part,**

1. 'the coordinator':

**ATHLONE INSTITUTE OF TECHNOLOGY (AIT)**, established in DUBLIN ROAD, ATHLONE, Ireland, represented for the purposes of signing the Agreement by Bill DELANEY

and the following other beneficiaries, if they sign their 'Accession Form' (see Annex 3 and Article 56):

2. **ACTECO PRODUCTOS Y SERVICIOS SL (ACTECO)**, established in C ZAMORA 24 POLIGONO INDUSTRIAL L ALFAC III, IBI ALICANTE 03440, Spain, VAT number: ESB03971512,

3. **AIMPLAS - ASOCIACION DE INVESTIGACION DE MATERIALES PLASTICOS Y CONEXAS (AIMPLAS)**, established in CALLE GUSTAVE EIFFEL 4 PARQUE TECNOLOGICO DE PATERNA, PATERNA VALENCIA 46980, Spain, VAT number: ESG46714853,

4. **AVECOM (AVECOM)**, established in INDUSTRIEWEG 122P, GENT-WONDELGEM 9032, Belgium, VAT number: BE0454894069,

5. **TECHNISCHE UNIVERSITAT CLAUSTHAL (TUC)**, established in ADOLPH ROMER STRASSE 2A, CLAUSTHAL ZELLERFELD 38678, Germany, VAT number: DE811282802,

6. **INSTITUT ZA MOLEKULARNU GENETIKU I GENETICKO INZENJERSTVO (IMGGE)**, established in VOJVODE STEPE 444A, BEOGRAD 11010, Serbia, VAT number: RS101736673,

7. **INSTITUTO DE BIOLOGIA EXPERIMENTAL E TECNOLÓGICA (IBET)**, established in AVENIDA DA REPUBLICA QUINTO DO MARQUES, OEIRAS 2781 901, Portugal, VAT number: PT502112255,

8. **LIMERICK INSTITUTE OF TECHNOLOGY (LIT)**, established in MOYLISH PARK, LIMERICK, Ireland, VAT number: IE6609432C,

9. **LOGOPLASTE INNOVATION LAB LDA (LOGOPLASTE)**, established in ESTRADA DA MALVEIRA ED LOGOPLASTE MATO ROMAO, CASCAIS 2750 782, Portugal, VAT number: PT505323354,

10. **MICROLIFE SOLUTIONS BV (MicroLife)**, established in SCIENCE PARK 406, AMSTERDAM 1098 XH, Netherlands, VAT number: NL850870938B01,

11. **NATIONAL TECHNICAL UNIVERSITY OF ATHENS - NTUA (NTUA)**, established in HEROON POLYTECHNIOU 9 ZOGRAPHOU CAMPUS, ATHINA 15780, Greece, VAT number: EL099793475,

12. **THE PROVOST, FELLOWS, FOUNDATION SCHOLARS & THE OTHER MEMBERS OF BOARD OF THE COLLEGE OF THE HOLY & UNDIVIDED TRINITY OF QUEEN ELIZABETH NEAR DUBLIN (TCD)**, established in College Green, DUBLIN 2, Ireland, VAT number: IE2200007U,

Unless otherwise specified, references to ‘beneficiary’ or ‘beneficiaries’ include the coordinator.

The parties referred to above have agreed to enter into the Agreement under the terms and conditions below.

By signing the Agreement or the Accession Form, the beneficiaries accept the grant and agree to implement it under their own responsibility and in accordance with the Agreement, with all the obligations and conditions it sets out.

The Agreement is composed of:

#### Terms and Conditions

Annex 1	Description of the action
Annex 2	Estimated budget for the action
	2a Additional information on the estimated budget
Annex 3	Accession Forms
Annex 4	Model for the financial statements
Annex 5	Model for the certificate on the financial statements
Annex 6	Model for the certificate on the methodology

# TERMS AND CONDITIONS

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## **CHAPTER 1 GENERAL**

### **ARTICLE 1 — SUBJECT OF THE AGREEMENT**

This Agreement sets out the rights and obligations and the terms and conditions applicable to the grant awarded to the beneficiaries for implementing the action set out in Chapter 2.

## **CHAPTER 2 ACTION**

### **ARTICLE 2 — ACTION TO BE IMPLEMENTED**

The grant is awarded for the action entitled ‘**Bio Innovation of a Circular Economy for Plastics**’ — ‘**BioICEP**’ (**‘action’**), as described in Annex 1.

### **ARTICLE 3 — DURATION AND STARTING DATE OF THE ACTION**

The duration of the action will be **48 months** as of 1 January 2020 (**‘starting date of the action’**).

### **ARTICLE 4 — ESTIMATED BUDGET AND BUDGET TRANSFERS**

#### **4.1 Estimated budget**

The ‘**estimated budget**’ for the action is set out in Annex 2.

It contains the estimated eligible costs and the forms of costs, broken down by beneficiary and budget category (see Articles 5, 6). It also shows the estimated costs of the international partners (see Article 14a).

#### **4.2 Budget transfers**

The estimated budget breakdown indicated in Annex 2 may be adjusted — without an amendment (see Article 55) — by transfers of amounts between beneficiaries, budget categories and/or forms of costs set out in Annex 2, if the action is implemented as described in Annex 1.

However, the beneficiaries may not add costs relating to subcontracts not provided for in Annex 1, unless such additional subcontracts are approved by an amendment or in accordance with Article 13.

## **CHAPTER 3 GRANT**

### **ARTICLE 5 — GRANT AMOUNT, FORM OF GRANT, REIMBURSEMENT RATES AND FORMS OF COSTS**

#### **5.1 Maximum grant amount**

The ‘**maximum grant amount**’ is **EUR 4 997 789.00** (four million nine hundred and ninety seven thousand seven hundred and eighty nine EURO).

#### **5.2 Form of grant, reimbursement rates and forms of costs**

The grant reimburses **100% of the action's eligible costs** (see Article 6) (**'reimbursement of eligible costs grant'**) (see Annex 2).

The estimated eligible costs of the action are EUR **4 997 791.25** (four million nine hundred and ninety seven thousand seven hundred and ninety one EURO and twenty five eurocents).

Eligible costs (see Article 6) must be declared under the following forms (**'forms of costs'**):

(a) for **direct personnel costs**:

- as actually incurred costs (**'actual costs'**) or
- on the basis of an amount per unit calculated by the beneficiary in accordance with its usual cost accounting practices (**'unit costs'**).

Personnel **costs for SME owners or beneficiaries that are natural persons** not receiving a salary (see Article 6.2, Points A.4 and A.5) must be declared on the basis of the amount per unit set out in Annex 2a (**unit costs**);

(b) for **direct costs for subcontracting**: as actually incurred costs (**actual costs**);

(c) for **direct costs of providing financial support to third parties**: not applicable;

(d) for **other direct costs**:

- for costs of internally invoiced goods and services: on the basis of an amount per unit calculated by the beneficiary in accordance with its usual cost accounting practices (**'unit costs'**);
- for all other costs: as actually incurred costs (**actual costs**);

(e) for **indirect costs**: on the basis of a flat-rate applied as set out in Article 6.2, Point E (**'flat-rate costs'**);

(f) **specific cost category(ies)**: not applicable.

### 5.3 Final grant amount — Calculation

The **'final grant amount'** depends on the actual extent to which the action is implemented in accordance with the Agreement's terms and conditions.

This amount is calculated by the Commission — when the payment of the balance is made (see Article 21.4) — in the following steps:

Step 1 — Application of the reimbursement rates to the eligible costs

Step 2 — Limit to the maximum grant amount

Step 3 — Reduction due to the no-profit rule

Step 4 — Reduction due to substantial errors, irregularities or fraud or serious breach of obligations



### 5.3.1 Step 1 — Application of the reimbursement rates to the eligible costs

The reimbursement rate(s) (see Article 5.2) are applied to the eligible costs (actual costs, unit costs and flat-rate costs; see Article 6) declared by the beneficiaries (see Article 20) and approved by the Commission (see Article 21).

### 5.3.2 Step 2 — Limit to the maximum grant amount

If the amount obtained following Step 1 is higher than the maximum grant amount set out in Article 5.1, it will be limited to the latter.

### 5.3.3 Step 3 — Reduction due to the no-profit rule

The grant must not produce a profit.

‘**Profit**’ means the surplus of the amount obtained following Steps 1 and 2 plus the action’s total receipts, over the action’s total eligible costs.

The ‘**action’s total eligible costs**’ are the consolidated total eligible costs approved by the Commission.

The ‘**action’s total receipts**’ are the consolidated total receipts generated during its duration (see Article 3).

The following are considered **receipts**:

- (a) income generated by the action; if the income is generated from selling equipment or other assets purchased under the Agreement, the receipt is up to the amount declared as eligible under the Agreement;
- (b) financial contributions given by third parties to the beneficiary specifically to be used for the action, and
- (c) in-kind contributions provided by third parties free of charge and specifically to be used for the action, if they have been declared as eligible costs.

The following are however not considered receipts:

- (a) income generated by exploiting the action’s results (see Article 28);
- (b) financial contributions by third parties, if they may be used to cover costs other than the eligible costs (see Article 6);
- (c) financial contributions by third parties with no obligation to repay any amount unused at the end of the period set out in Article 3.

If there is a profit, it will be deducted from the amount obtained following Steps 1 and 2.

### 5.3.4 Step 4 — Reduction due to substantial errors, irregularities or fraud or serious breach of obligations — Reduced grant amount — Calculation

If the grant is reduced (see Article 43), the Commission will calculate the reduced grant amount by deducting the amount of the reduction (calculated in proportion to the seriousness of the errors,

irregularities or fraud or breach of obligations, in accordance with Article 43.2) from the maximum grant amount set out in Article 5.1.

The final grant amount will be the lower of the following two:

- the amount obtained following Steps 1 to 3 or
- the reduced grant amount following Step 4.

#### 5.4 Revised final grant amount — Calculation

If — after the payment of the balance (in particular, after checks, reviews, audits or investigations; see Article 22) — the Commission rejects costs (see Article 42) or reduces the grant (see Article 43), it will calculate the ‘**revised final grant amount**’ for the beneficiary concerned by the findings.

This amount is calculated by the Commission on the basis of the findings, as follows:

- in case of **rejection of costs**: by applying the reimbursement rate to the revised eligible costs approved by the Commission for the beneficiary concerned;
- in case of **reduction of the grant**: by calculating the concerned beneficiary’s share in the grant amount reduced in proportion to the seriousness of the errors, irregularities or fraud or breach of obligations (see Article 43.2).

In case of **rejection of costs and reduction of the grant**, the revised final grant amount for the beneficiary concerned will be the lower of the two amounts above.

## ARTICLE 6 — ELIGIBLE AND INELIGIBLE COSTS

### 6.1 General conditions for costs to be eligible

‘**Eligible costs**’ are costs that meet the following criteria:

(a) for **actual costs**:

- (i) they must be actually incurred by the beneficiary;
- (ii) they must be incurred in the period set out in Article 3, with the exception of costs relating to the submission of the periodic report for the last reporting period and the final report (see Article 20);
- (iii) they must be indicated in the estimated budget set out in Annex 2;
- (iv) they must be incurred in connection with the action as described in Annex 1 and necessary for its implementation;
- (v) they must be identifiable and verifiable, in particular recorded in the beneficiary’s accounts in accordance with the accounting standards applicable in the country where the beneficiary is established and with the beneficiary’s usual cost accounting practices;
- (vi) they must comply with the applicable national law on taxes, labour and social security, and

- (vii) they must be reasonable, justified and must comply with the principle of sound financial management, in particular regarding economy and efficiency;

(b) for **unit costs**:

- (i) they must be calculated as follows:

{amounts per unit set out in Annex 2a or calculated by the beneficiary in accordance with its usual cost accounting practices (see Article 6.2, Point A and Article 6.2.D.5)}

multiplied by

the number of actual units};

- (ii) the number of actual units must comply with the following conditions:

- the units must be actually used or produced in the period set out in Article 3;
- the units must be necessary for implementing the action or produced by it, and
- the number of units must be identifiable and verifiable, in particular supported by records and documentation (see Article 18);

(c) for **flat-rate costs**:

- (i) they must be calculated by applying the flat-rate set out in Annex 2, and
- (ii) the costs (actual costs or unit costs) to which the flat-rate is applied must comply with the conditions for eligibility set out in this Article.

## 6.2 Specific conditions for costs to be eligible

Costs are eligible if they comply with the general conditions (see above) and the specific conditions set out below for each of the following budget categories:

- A. direct personnel costs;
- B. direct costs of subcontracting;
- C. not applicable;
- D. other direct costs;
- E. indirect costs;
- F. not applicable.

‘Direct costs’ are costs that are directly linked to the action implementation and can therefore be attributed to it directly. They must not include any indirect costs (see Point E below).

‘Indirect costs’ are costs that are not directly linked to the action implementation and therefore cannot be attributed directly to it.

### A. Direct personnel costs

#### Types of eligible personnel costs

A.1 Personnel costs are eligible, if they are related to personnel working for the beneficiary under an employment contract (or equivalent appointing act) and assigned to the action (**‘costs for employees (or equivalent)’**). They must be limited to salaries (including during parental leave), social security contributions, taxes and other costs included in the **remuneration**, if they arise from national law or the employment contract (or equivalent appointing act).

Beneficiaries that are non-profit legal entities<sup>1</sup> may also declare as personnel costs **additional remuneration** for personnel assigned to the action (including payments on the basis of supplementary contracts regardless of their nature), if:

- (a) it is part of the beneficiary’s usual remuneration practices and is paid in a consistent manner whenever the same kind of work or expertise is required;
- (b) the criteria used to calculate the supplementary payments are objective and generally applied by the beneficiary, regardless of the source of funding used.

‘Additional remuneration’ means any part of the remuneration which exceeds what the person would be paid for time worked in projects funded by national schemes.

Additional remuneration for personnel assigned to the action is eligible up to the following amount:

- (a) if the person works full time and exclusively on the action during the full year: up to EUR 8 000;
- (b) if the person works exclusively on the action but not full-time or not for the full year: up to the corresponding pro-rata amount of EUR 8 000, or
- (c) if the person does not work exclusively on the action: up to a pro-rata amount calculated as follows:
 
$$\left\{ \begin{array}{l} \text{EUR 8 000} \\ \text{divided by} \\ \text{the number of annual productive hours (see below)}, \\ \text{multiplied by} \\ \text{the number of hours that the person has worked on the action during the year} \end{array} \right\}.$$

A.2 The **costs for natural persons working under a direct contract** with the beneficiary other than an employment contract are eligible personnel costs, if:

- (a) the person works under conditions similar to those of an employee (in particular regarding the way the work is organised, the tasks that are performed and the premises where they are performed);
- (b) the result of the work carried out belongs to the beneficiary (unless exceptionally agreed otherwise), and

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<sup>1</sup> For the definition, see Article 2.1(14) of the Rules for Participation Regulation No 1290/2013: ‘**non-profit legal entity**’ means a legal entity which by its legal form is non-profit-making or which has a legal or statutory obligation not to distribute profits to its shareholders or individual members.

- (c) the costs are not significantly different from those for personnel performing similar tasks under an employment contract with the beneficiary.

A.3 The **costs of personnel seconded by a third party against payment** are eligible personnel costs, if the conditions in Article 11.1 are met.

A.4 **Costs of owners** of beneficiaries that are small and medium-sized enterprises ('**SME owners**') who are working on the action and who do not receive a salary are eligible personnel costs, if they correspond to the amount per unit set out in Annex 2a multiplied by the number of actual hours worked on the action.

A.5 **Costs of 'beneficiaries that are natural persons'** not receiving a salary are eligible personnel costs, if they correspond to the amount per unit set out in Annex 2a multiplied by the number of actual hours worked on the action.

### Calculation

Personnel costs must be calculated by the beneficiaries as follows:

{ {hourly rate  
multiplied by  
the number of actual hours worked on the action},  
plus  
for non-profit legal entities: additional remuneration to personnel assigned to the action under the  
conditions set out above (Point A.1)}.

The number of actual hours declared for a person must be identifiable and verifiable (see Article 18).

The total number of hours declared in EU or Euratom grants, for a person for a year, cannot be higher than the annual productive hours used for the calculations of the hourly rate. Therefore, the maximum number of hours that can be declared for the grant are:

{number of annual productive hours for the year (see below)  
minus  
total number of hours declared by the beneficiary, for that person in that year, for other EU or Euratom  
grants}.

The '**hourly rate**' is one of the following:

- (a) for personnel costs declared as **actual costs** (i.e. budget categories A.1, A.2, A.3): the hourly rate is calculated *per full financial year*, as follows:

{actual annual personnel costs (excluding additional remuneration) for the person  
divided by  
number of annual productive hours}.

using the personnel costs and the number of productive hours for each full financial year covered by the reporting period concerned. If a financial year is not closed at the end of the

reporting period, the beneficiaries must use the hourly rate of the last closed financial year available.

For the ‘number of annual productive hours’, the beneficiaries may choose one of the following:

- (i) ‘fixed number of hours’: 1 720 hours for persons working full time (or corresponding pro-rata for persons not working full time);
- (ii) ‘individual annual productive hours’: the total number of hours worked by the person in the year for the beneficiary, calculated as follows:

{annual workable hours of the person (according to the employment contract, applicable collective labour agreement or national law)

plus

overtime worked

minus

absences (such as sick leave and special leave)}.

‘Annual workable hours’ means the period during which the personnel must be working, at the employer’s disposal and carrying out his/her activity or duties under the employment contract, applicable collective labour agreement or national working time legislation.

If the contract (or applicable collective labour agreement or national working time legislation) does not allow to determine the annual workable hours, this option cannot be used;

- (iii) ‘standard annual productive hours’: the ‘standard number of annual hours’ generally applied by the beneficiary for its personnel in accordance with its usual cost accounting practices. This number must be at least 90% of the ‘standard annual workable hours’.

If there is no applicable reference for the standard annual workable hours, this option cannot be used.

For all options, the actual time spent on **parental leave** by a person assigned to the action may be deducted from the number of annual productive hours.

As an alternative, beneficiaries may calculate the hourly rate *per month*, as follows:

{actual monthly personnel cost (excluding additional remuneration) for the person

divided by

{number of annual productive hours / 12}}}

using the personnel costs for each month and (one twelfth of) the annual productive hours calculated according to either option (i) or (iii) above, i.e.:

- fixed number of hours or
- standard annual productive hours.

Time spent on **parental leave** may not be deducted when calculating the hourly rate per month. However, beneficiaries may declare personnel costs incurred in periods of parental leave in proportion to the time the person worked on the action in that financial year.

If parts of a basic remuneration are generated over a period longer than a month, the beneficiaries may include only the share which is generated in the month (irrespective of the amount actually paid for that month).

Each beneficiary must use only one option (per full financial year or per month) for each full financial year;

(b) for personnel costs declared on the basis of **unit costs** (i.e. budget categories A.1, A.2, A.4, A.5): the hourly rate is one of the following:

- (i) for SME owners or beneficiaries that are natural persons: the hourly rate set out in Annex 2a (see Points A.4 and A.5 above), or
- (ii) for personnel costs declared on the basis of the beneficiary's usual cost accounting practices: the hourly rate calculated by the beneficiary in accordance with its usual cost accounting practices, if:
  - the cost accounting practices used are applied in a consistent manner, based on objective criteria, regardless of the source of funding;
  - the hourly rate is calculated using the actual personnel costs recorded in the beneficiary's accounts, excluding any ineligible cost or costs included in other budget categories.

The actual personnel costs may be adjusted by the beneficiary on the basis of budgeted or estimated elements. Those elements must be relevant for calculating the personnel costs, reasonable and correspond to objective and verifiable information;

and

- the hourly rate is calculated using the number of annual productive hours (see above).

**B. Direct costs of subcontracting** (including related duties, taxes and charges such as non-deductible value added tax (VAT) paid by the beneficiary) are eligible if the conditions in Article 13.1.1 are met.

**C. Direct costs of providing financial support to third parties**

Not applicable

**D. Other direct costs**

**D.1 Travel costs and related subsistence allowances** (including related duties, taxes and charges such as non-deductible value added tax (VAT) paid by the beneficiary) are eligible if they are in line with the beneficiary's usual practices on travel.

**D.2 The depreciation costs of equipment, infrastructure or other assets** (new or second-hand) as recorded in the beneficiary's accounts are eligible, if they were purchased in accordance with



Article 10.1.1 and written off in accordance with international accounting standards and the beneficiary's usual accounting practices.

The **costs of renting or leasing** equipment, infrastructure or other assets (including related duties, taxes and charges such as non-deductible value added tax (VAT) paid by the beneficiary) are also eligible, if they do not exceed the depreciation costs of similar equipment, infrastructure or assets and do not include any financing fees.

The costs of equipment, infrastructure or other assets **contributed in-kind against payment** are eligible, if they do not exceed the depreciation costs of similar equipment, infrastructure or assets, do not include any financing fees and if the conditions in Article 11.1 are met.

The only portion of the costs that will be taken into account is that which corresponds to the duration of the action and rate of actual use for the purposes of the action.

**D.3 Costs of other goods and services** (including related duties, taxes and charges such as non-deductible value added tax (VAT) paid by the beneficiary) are eligible, if they are:

- (a) purchased specifically for the action and in accordance with Article 10.1.1 or
- (b) contributed in kind against payment and in accordance with Article 11.1.

Such goods and services include, for instance, consumables and supplies, dissemination (including open access), protection of results, certificates on the financial statements (if they are required by the Agreement), certificates on the methodology, translations and publications.

**D.4 Capitalised and operating costs of 'large research infrastructure'**<sup>2</sup> directly used for the action are eligible, if:

- (a) the value of the large research infrastructure represents at least 75% of the total fixed assets (at historical value in its last closed balance sheet before the date of the signature of the Agreement or as determined on the basis of the rental and leasing costs of the research infrastructure<sup>3</sup>);
- (b) the beneficiary's methodology for declaring the costs for large research infrastructure has been positively assessed by the Commission ('**ex-ante assessment**');
- (c) the beneficiary declares as direct eligible costs only the portion which corresponds to the duration of the action and the rate of actual use for the purposes of the action, and
- (d) they comply with the conditions as further detailed in the annotations to the H2020 grant agreements.

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<sup>2</sup> '**Large research infrastructure**' means research infrastructure of a total value of at least EUR 20 million, for a beneficiary, calculated as the sum of historical asset values of each individual research infrastructure of that beneficiary, as they appear in its last closed balance sheet before the date of the signature of the Agreement or as determined on the basis of the rental and leasing costs of the research infrastructure.

<sup>3</sup> For the definition, see Article 2(6) of the H2020 Framework Programme Regulation No 1291/2013: '**Research infrastructure**' are facilities, resources and services that are used by the research communities to conduct research and foster innovation in their fields. Where relevant, they may be used beyond research, e.g. for education or public services. They include: major scientific equipment (or sets of instruments); knowledge-based resources such as collections, archives or scientific data; e-infrastructures such as data and computing systems and communication networks; and any other infrastructure of a unique nature essential to achieve excellence in research and innovation. Such infrastructures may be 'single-sited', 'virtual' or 'distributed'.



#### D.5 Costs of internally invoiced goods and services directly used for the action are eligible, if:

- (a) they are declared on the basis of a unit cost calculated in accordance with the beneficiary's usual cost accounting practices;
- (b) the cost accounting practices used are applied in a consistent manner, based on objective criteria, regardless of the source of funding;
- (c) the unit cost is calculated using the actual costs for the good or service recorded in the beneficiary's accounts, excluding any ineligible cost or costs included in other budget categories.

The actual costs may be adjusted by the beneficiary on the basis of budgeted or estimated elements. Those elements must be relevant for calculating the costs, reasonable and correspond to objective and verifiable information;

- (d) the unit cost excludes any costs of items which are not directly linked to the production of the invoiced goods or service.

'Internally invoiced goods and services' means goods or services which are provided by the beneficiary directly for the action and which the beneficiary values on the basis of its usual cost accounting practices.

#### E. Indirect costs

**Indirect costs** are eligible if they are declared on the basis of the flat-rate of 25% of the eligible direct costs (see Article 5.2 and Points A to D above), from which are excluded:

- (a) costs of subcontracting and
- (b) costs of in-kind contributions provided by third parties which are not used on the beneficiary's premises;
- (c) not applicable;
- (d) not applicable.

Beneficiaries receiving an operating grant<sup>4</sup> financed by the EU or Euratom budget cannot declare indirect costs for the period covered by the operating grant, unless they can demonstrate that the operating grant does not cover any costs of the action.

#### F. Specific cost category(ies)

Not applicable

### 6.3 Conditions for costs of linked third parties to be eligible

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<sup>4</sup> For the definition, see Article 121(1)(b) of Regulation (EU, Euratom) No 966/2012 of the European Parliament and of the Council of 25 October 2012 on the financial rules applicable to the general budget of the Union and repealing Council Regulation (EC, Euratom) No 1605/2002 ('**Financial Regulation No 966/2012**') (OJ L 218, 26.10.2012, p.1): '**operating grant**' means direct financial contribution, by way of donation, from the budget in order to finance the functioning of a body which pursues an aim of general EU interest or has an objective forming part of and supporting an EU policy.

Not applicable

#### **6.4 Conditions for in-kind contributions provided by third parties free of charge to be eligible**

**In-kind contributions provided free of charge** are eligible direct costs (for the beneficiary), if the costs incurred by the third party fulfil — *mutatis mutandis* — the general and specific conditions for eligibility set out in this Article (Article 6.1 and 6.2) and Article 12.1.

#### **6.5 Ineligible costs**

‘**Ineligible costs**’ are:

- (a) costs that do not comply with the conditions set out above (Article 6.1 to 6.4), in particular:
  - (i) costs related to return on capital;
  - (ii) debt and debt service charges;
  - (iii) provisions for future losses or debts;
  - (iv) interest owed;
  - (v) doubtful debts;
  - (vi) currency exchange losses;
  - (vii) bank costs charged by the beneficiary’s bank for transfers from the Commission;
  - (viii) excessive or reckless expenditure;
  - (ix) deductible VAT;
  - (x) costs incurred during suspension of the implementation of the action (see Article 49);
- (b) costs declared under another EU or Euratom grant (including grants awarded by a Member State and financed by the EU or Euratom budget and grants awarded by bodies other than the Commission for the purpose of implementing the EU or Euratom budget); in particular, indirect costs if the beneficiary is already receiving an operating grant financed by the EU or Euratom budget in the same period, unless it can demonstrate that the operating grant does not cover any costs of the action.

#### **6.6 Consequences of declaration of ineligible costs**

Declared costs that are ineligible will be rejected (see Article 42).

This may also lead to any of the other measures described in Chapter 6.

### **CHAPTER 4 RIGHTS AND OBLIGATIONS OF THE PARTIES**

## **SECTION 1 RIGHTS AND OBLIGATIONS RELATED TO IMPLEMENTING THE ACTION**

### **ARTICLE 7 — GENERAL OBLIGATION TO PROPERLY IMPLEMENT THE ACTION**

#### **7.1 General obligation to properly implement the action**

The beneficiaries must implement the action as described in Annex 1 and in compliance with the provisions of the Agreement and all legal obligations under applicable EU, international and national law.

#### **7.2 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

### **ARTICLE 8 — RESOURCES TO IMPLEMENT THE ACTION — THIRD PARTIES INVOLVED IN THE ACTION**

The beneficiaries must have the appropriate resources to implement the action.

If it is necessary to implement the action, the beneficiaries may:

- purchase goods, works and services (see Article 10);
- use in-kind contributions provided by third parties against payment (see Article 11);
- use in-kind contributions provided by third parties free of charge (see Article 12);
- call upon subcontractors to implement action tasks described in Annex 1 (see Article 13);
- call upon linked third parties to implement action tasks described in Annex 1 (see Article 14);
- call upon international partners to implement action tasks described in Annex 1 (see Article 14a).

In these cases, the beneficiaries retain sole responsibility towards the Commission and the other beneficiaries for implementing the action.

### **ARTICLE 9 — IMPLEMENTATION OF ACTION TASKS BY BENEFICIARIES NOT RECEIVING EU FUNDING**

Not applicable

### **ARTICLE 10 — PURCHASE OF GOODS, WORKS OR SERVICES**

#### **10.1 Rules for purchasing goods, works or services**

10.1.1 If necessary to implement the action, the beneficiaries may purchase goods, works or services.

The beneficiaries must make such purchases ensuring the best value for money or, if appropriate, the lowest price. In doing so, they must avoid any conflict of interests (see Article 35).

The beneficiaries must ensure that the Commission, the European Court of Auditors (ECA) and the European Anti-Fraud Office (OLAF) can exercise their rights under Articles 22 and 23 also towards their contractors.

10.1.2 Beneficiaries that are ‘contracting authorities’ within the meaning of Directive 2004/18/EC<sup>5</sup> (or 2014/24/EU<sup>6</sup>) or ‘contracting entities’ within the meaning of Directive 2004/17/EC<sup>7</sup> (or 2014/25/EU<sup>8</sup>) must comply with the applicable national law on public procurement.

## **10.2 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under Article 10.1.1, the costs related to the contract concerned will be ineligible (see Article 6) and will be rejected (see Article 42).

If a beneficiary breaches any of its obligations under Article 10.1.2, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

## **ARTICLE 11 — USE OF IN-KIND CONTRIBUTIONS PROVIDED BY THIRD PARTIES AGAINST PAYMENT**

### **11.1 Rules for the use of in-kind contributions against payment**

If necessary to implement the action, the beneficiaries may use in-kind contributions provided by third parties against payment.

The beneficiaries may declare costs related to the payment of in-kind contributions as eligible (see Article 6.1 and 6.2), up to the third parties’ costs for the seconded persons, contributed equipment, infrastructure or other assets or other contributed goods and services.

The third parties and their contributions must be set out in Annex 1. The Commission may however approve in-kind contributions not set out in Annex 1 without amendment (see Article 55), if:

- they are specifically justified in the periodic technical report and
- their use does not entail changes to the Agreement which would call into question the decision awarding the grant or breach the principle of equal treatment of applicants.

The beneficiaries must ensure that the Commission, the European Court of Auditors (ECA) and the

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<sup>5</sup> Directive 2004/18/EC of the European Parliament and of the Council of 31 March 2004 on the coordination of procedures for the award of public work contracts, public supply contracts and public service contracts (OJ L 134, 30.04.2004, p. 114).

<sup>6</sup> Directive 2014/24/EU of the European Parliament and of the Council of 26 February 2014 on public procurement and repealing Directive 2004/18/EC. (OJ L 94, 28.03.2014, p. 65).

<sup>7</sup> Directive 2004/17/EC of the European Parliament and of the Council of 31 March 2004 coordinating the procurement procedures of entities operating in the water, energy, transport and postal services sectors (OJ L 134, 30.04.2004, p. 1)

<sup>8</sup> Directive 2014/25/EU of the European Parliament and of the Council of 26 February 2014 on procurement by entities operating in the water, energy, transport and postal services sectors and repealing Directive 2004/17/EC (OJ L 94, 28.03.2014, p. 243).

European Anti-Fraud Office (OLAF) can exercise their rights under Articles 22 and 23 also towards the third parties.

## **11.2 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the costs related to the payment of the in-kind contribution will be ineligible (see Article 6) and will be rejected (see Article 42).

Such breaches may also lead to any of the other measures described in Chapter 6.

## **ARTICLE 12 — USE OF IN-KIND CONTRIBUTIONS PROVIDED BY THIRD PARTIES FREE OF CHARGE**

### **12.1 Rules for the use of in-kind contributions free of charge**

If necessary to implement the action, the beneficiaries may use in-kind contributions provided by third parties free of charge.

The beneficiaries may declare costs incurred by the third parties for the seconded persons, contributed equipment, infrastructure or other assets or other contributed goods and services as eligible in accordance with Article 6.4.

The third parties and their contributions must be set out in Annex 1. The Commission may however approve in-kind contributions not set out in Annex 1 without amendment (see Article 55), if:

- they are specifically justified in the periodic technical report and
- their use does not entail changes to the Agreement which would call into question the decision awarding the grant or breach the principle of equal treatment of applicants.

The beneficiaries must ensure that the Commission, the European Court of Auditors (ECA) and the European Anti-Fraud Office (OLAF) can exercise their rights under Articles 22 and 23 also towards the third parties.

### **12.2 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the costs incurred by the third parties related to the in-kind contribution will be ineligible (see Article 6) and will be rejected (see Article 42).

Such breaches may also lead to any of the other measures described in Chapter 6.

## **ARTICLE 13 — IMPLEMENTATION OF ACTION TASKS BY SUBCONTRACTORS**

### **13.1 Rules for subcontracting action tasks**

13.1.1 If necessary to implement the action, the beneficiaries may award subcontracts covering the implementation of certain action tasks described in Annex 1.

Subcontracting may cover only a limited part of the action.

The beneficiaries must award the subcontracts ensuring the best value for money or, if appropriate, the lowest price. In doing so, they must avoid any conflict of interests (see Article 35).

The tasks to be implemented and the estimated cost for each subcontract must be set out in Annex 1 and the total estimated costs of subcontracting per beneficiary must be set out in Annex 2. The Commission may however approve subcontracts not set out in Annex 1 and 2 without amendment (see Article 55), if:

- they are specifically justified in the periodic technical report and
- they do not entail changes to the Agreement which would call into question the decision awarding the grant or breach the principle of equal treatment of applicants.

The beneficiaries must ensure that the Commission, the European Court of Auditors (ECA) and the European Anti-Fraud Office (OLAF) can exercise their rights under Articles 22 and 23 also towards their subcontractors.

13.1.2 The beneficiaries must ensure that their obligations under Articles 35, 36, 38 and 46 also apply to the subcontractors.

Beneficiaries that are ‘contracting authorities’ within the meaning of Directive 2004/18/EC (or 2014/24/EU) or ‘contracting entities’ within the meaning of Directive 2004/17/EC (or 2014/25/EU) must comply with the applicable national law on public procurement.

## **13.2 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under Article 13.1.1, the costs related to the subcontract concerned will be ineligible (see Article 6) and will be rejected (see Article 42).

If a beneficiary breaches any of its obligations under Article 13.1.2, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

## **ARTICLE 14 — IMPLEMENTATION OF ACTION TASKS BY LINKED THIRD PARTIES**

Not applicable

## **ARTICLE 14a — IMPLEMENTATION OF ACTION TASKS BY INTERNATIONAL PARTNERS**

### **14a.1 Rules for calling upon international partners to implement part of the action**

The following **international partners**<sup>12</sup> may implement the action tasks attributed to them in Annex 1:

- Institute of Microbiology, Chinese Academy of Sciences (IMCAS), international partner of AIT
- SHANDONG UNIVERSITY (SU), international partner of AIT
- Beijing Institute of Technology (BIT), international partner of AIT

The costs of the international partners are estimated in Annex 2 but:

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<sup>12</sup> ‘International partner’ is any legal entity established in a non-associated third country which is not eligible for funding under Article 10 of the Rules for Participation Regulation No 1290/2013.

- will not be reimbursed and
- will not be taken into account for the calculation of the grant.

The beneficiaries must ensure that the Commission, the European Court of Auditors (ECA) and the European Anti-Fraud Office (OLAF) can exercise their rights under Articles 22 and 23 also towards their international partners.

The beneficiaries must ensure that their obligations under Articles 18.1.1, 20.3(a), 20.4(a), 35, 36, 38 also apply to their international partners.

#### **14a.2 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

### **ARTICLE 15 — FINANCIAL SUPPORT TO THIRD PARTIES**

#### **15.1 Rules for providing financial support to third parties**

Not applicable

#### **15.2 Financial support in the form of prizes**

Not applicable

#### **15.3 Consequences of non-compliance**

Not applicable

### **ARTICLE 16 — PROVISION OF TRANS-NATIONAL OR VIRTUAL ACCESS TO RESEARCH INFRASTRUCTURE**

#### **16.1 Rules for providing trans-national access to research infrastructure**

Not applicable

#### **16.2 Rules for providing virtual access to research infrastructure**

Not applicable

#### **16.3 Consequences of non-compliance**

Not applicable

## **SECTION 2 RIGHTS AND OBLIGATIONS RELATED TO THE GRANT ADMINISTRATION**

## ARTICLE 17 — GENERAL OBLIGATION TO INFORM

### 17.1 General obligation to provide information upon request

The beneficiaries must provide — during implementation of the action or afterwards and in accordance with Article 41.2 — any information requested in order to verify eligibility of the costs, proper implementation of the action and compliance with any other obligation under the Agreement.

### 17.2 Obligation to keep information up to date and to inform about events and circumstances likely to affect the Agreement

Each beneficiary must keep information stored in the Participant Portal Beneficiary Register (via the electronic exchange system; see Article 52) up to date, in particular, its name, address, legal representatives, legal form and organisation type.

Each beneficiary must immediately inform the coordinator — which must immediately inform the Commission and the other beneficiaries — of any of the following:

- (a) **events** which are likely to affect significantly or delay the implementation of the action or the EU's financial interests, in particular:
  - (i) changes in its legal, financial, technical, organisational or ownership situation
- (b) **circumstances** affecting:
  - (i) the decision to award the grant or
  - (ii) compliance with requirements under the Agreement.

### 17.3 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

## ARTICLE 18 — KEEPING RECORDS — SUPPORTING DOCUMENTATION

### 18.1 Obligation to keep records and other supporting documentation

The beneficiaries must — for a period of five years after the payment of the balance — keep records and other supporting documentation in order to prove the proper implementation of the action and the costs they declare as eligible.

They must make them available upon request (see Article 17) or in the context of checks, reviews, audits or investigations (see Article 22).

If there are on-going checks, reviews, audits, investigations, litigation or other pursuits of claims under the Agreement (including the extension of findings; see Article 22), the beneficiaries must keep the records and other supporting documentation until the end of these procedures.

The beneficiaries must keep the original documents. Digital and digitalised documents are considered



originals if they are authorised by the applicable national law. The Commission may accept non-original documents if it considers that they offer a comparable level of assurance.

### 18.1.1 Records and other supporting documentation on the scientific and technical implementation

The beneficiaries must keep records and other supporting documentation on scientific and technical implementation of the action in line with the accepted standards in the respective field.

### 18.1.2 Records and other documentation to support the costs declared

The beneficiaries must keep the records and documentation supporting the costs declared, in particular the following:

- (a) for **actual costs**: adequate records and other supporting documentation to prove the costs declared, such as contracts, subcontracts, invoices and accounting records. In addition, the beneficiaries' usual cost accounting practices and internal control procedures must enable direct reconciliation between the amounts declared, the amounts recorded in their accounts and the amounts stated in the supporting documentation;
- (b) for **unit costs**: adequate records and other supporting documentation to prove the number of units declared. Beneficiaries do not need to identify the actual eligible costs covered or to keep or provide supporting documentation (such as accounting statements) to prove the amount per unit.

In addition, **for unit costs calculated in accordance with the beneficiary's usual cost accounting practices**, the beneficiaries must keep adequate records and documentation to prove that the cost accounting practices used comply with the conditions set out in Article 6.2.

The beneficiaries may submit to the Commission, for approval, a certificate (drawn up in accordance with Annex 6) stating that their usual cost accounting practices comply with these conditions (**'certificate on the methodology'**). If the certificate is approved, costs declared in line with this methodology will not be challenged subsequently, unless the beneficiaries have concealed information for the purpose of the approval.

- (c) for **flat-rate costs**: adequate records and other supporting documentation to prove the eligibility of the costs to which the flat-rate is applied. The beneficiaries do not need to identify the costs covered or provide supporting documentation (such as accounting statements) to prove the amount declared at a flat-rate.

In addition, for **personnel costs** (declared as actual costs or on the basis of unit costs), the beneficiaries must keep **time records** for the number of hours declared. The time records must be in writing and approved by the persons working on the action and their supervisors, at least monthly. In the absence of reliable time records of the hours worked on the action, the Commission may accept alternative evidence supporting the number of hours declared, if it considers that it offers an adequate level of assurance.

As an exception, for **persons working exclusively on the action**, there is no need to keep time records, if the beneficiary signs a **declaration** confirming that the persons concerned have worked exclusively on the action.

## 18.2 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, costs insufficiently substantiated will be ineligible (see Article 6) and will be rejected (see Article 42), and the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

## ARTICLE 19 — SUBMISSION OF DELIVERABLES

### 19.1 Obligation to submit deliverables

The coordinator must submit the ‘**deliverables**’ identified in Annex 1, in accordance with the timing and conditions set out in it.

### 19.2 Consequences of non-compliance

If the coordinator breaches any of its obligations under this Article, the Commission may apply any of the measures described in Chapter 6.

## ARTICLE 20 — REPORTING — PAYMENT REQUESTS

### 20.1 Obligation to submit reports

The coordinator must submit to the Commission (see Article 52) the technical and financial reports set out in this Article. These reports include requests for payment and must be drawn up using the forms and templates provided in the electronic exchange system (see Article 52).

### 20.2 Reporting periods

The action is divided into the following ‘**reporting periods**’:

- RP1: from month 1 to month 12
- RP2: from month 13 to month 24
- RP3: from month 25 to month 36
- RP4: from month 37 to month 48

### 20.3 Periodic reports — Requests for interim payments

The coordinator must submit a periodic report within 60 days following the end of each reporting period.

The **periodic report** must include the following:

(a) a ‘**periodic technical report**’ containing:

- (i) an **explanation of the work carried out** by the beneficiaries;
- (ii) an **overview of the progress** towards the objectives of the action, including milestones and deliverables identified in Annex 1.

This report must include explanations justifying the differences between work expected to be carried out in accordance with Annex 1 and that actually carried out.

The report must detail the exploitation and dissemination of the results and — if required in Annex 1 — an updated '**plan for the exploitation and dissemination of the results**'.

The report must indicate the communication activities;

- (iii) a **summary** for publication by the Commission;
  - (iv) the answers to the '**questionnaire**', covering issues related to the action implementation and the economic and societal impact, notably in the context of the Horizon 2020 key performance indicators and the Horizon 2020 monitoring requirements;
- (b) a '**periodic financial report**' containing:

- (i) an '**individual financial statement**' (see Annex 4) from each beneficiary, for the reporting period concerned.

The individual financial statement must detail the eligible costs (actual costs, unit costs and flat-rate costs; see Article 6) for each budget category (see Annex 2).

The beneficiaries must declare all eligible costs, even if — for actual costs, unit costs and flat-rate costs — they exceed the amounts indicated in the estimated budget (see Annex 2). Amounts which are not declared in the individual financial statement will not be taken into account by the Commission.

If an individual financial statement is not submitted for a reporting period, it may be included in the periodic financial report for the next reporting period.

The individual financial statements of the last reporting period must also detail the **receipts of the action** (see Article 5.3.3).

Each beneficiary must **certify** that:

- the information provided is full, reliable and true;
  - the costs declared are eligible (see Article 6);
  - the costs can be substantiated by adequate records and supporting documentation (see Article 18) that will be produced upon request (see Article 17) or in the context of checks, reviews, audits and investigations (see Article 22), and
  - for the last reporting period: that all the receipts have been declared (see Article 5.3.3);
- (ii) an **explanation of the use of resources** and the information on subcontracting (see Article 13) and in-kind contributions provided by third parties (see Articles 11 and 12) from each beneficiary, for the reporting period concerned;
  - (iii) not applicable;

- (iv) a '**periodic summary financial statement**', created automatically by the electronic exchange system, consolidating the individual financial statements for the reporting period concerned and including — except for the last reporting period — the **request for interim payment**.

#### 20.4 Final report — Request for payment of the balance

In addition to the periodic report for the last reporting period, the coordinator must submit the final report within 60 days following the end of the last reporting period.

The **final report** must include the following:

- (a) a '**final technical report**' with a **summary** for publication containing:
  - (i) an overview of the results and their exploitation and dissemination;
  - (ii) the conclusions on the action, and
  - (iii) the socio-economic impact of the action;
- (b) a '**final financial report**' containing:
  - (i) a '**final summary financial statement**', created automatically by the electronic exchange system, consolidating the individual financial statements for all reporting periods and including the **request for payment of the balance** and
  - (ii) a '**certificate on the financial statements**' (drawn up in accordance with Annex 5) for each beneficiary, if it requests a total contribution of EUR 325 000 or more, as reimbursement of actual costs and unit costs calculated on the basis of its usual cost accounting practices (see Article 5.2 and Article 6.2).

#### 20.5 Information on cumulative expenditure incurred

Not applicable

#### 20.6 Currency for financial statements and conversion into euro

Financial statements must be drafted in euro.

Beneficiaries with accounting established in a currency other than the euro must convert the costs recorded in their accounts into euro, at the average of the daily exchange rates published in the C series of the *Official Journal of the European Union*, calculated over the corresponding reporting period.

If no daily euro exchange rate is published in the *Official Journal of the European Union* for the currency in question, they must be converted at the average of the monthly accounting rates published on the Commission's website, calculated over the corresponding reporting period.

Beneficiaries with accounting established in euro must convert costs incurred in another currency into euro according to their usual accounting practices.

#### 20.7 Language of reports

All reports (technical and financial reports, including financial statements) must be submitted in the language of the Agreement.

## 20.8 Consequences of non-compliance

If the reports submitted do not comply with this Article, the Commission may suspend the payment deadline (see Article 47) and apply any of the other measures described in Chapter 6.

If the coordinator breaches its obligation to submit the reports and if it fails to comply with this obligation within 30 days following a written reminder, the Commission may terminate the Agreement (see Article 50) or apply any of the other measures described in Chapter 6.

## ARTICLE 21 — PAYMENTS AND PAYMENT ARRANGEMENTS

### 21.1 Payments to be made

The following payments will be made to the coordinator:

- one **pre-financing payment**;
- one or more **interim payments**, on the basis of the request(s) for interim payment (see Article 20), and
- one **payment of the balance**, on the basis of the request for payment of the balance (see Article 20).

### 21.2 Pre-financing payment — Amount — Amount retained for the Guarantee Fund

The aim of the pre-financing is to provide the beneficiaries with a float.

It remains the property of the EU until the payment of the balance.

The amount of the pre-financing payment will be EUR **1 999 115.60** (one million nine hundred and ninety nine thousand one hundred and fifteen EURO and sixty eurocents).

The Commission will — except if Article 48 applies — make the pre-financing payment to the coordinator within 30 days, either from the entry into force of the Agreement (see Article 58) or from 10 days before the starting date of the action (see Article 3), whichever is the latest.

An amount of EUR **249 889.45** (two hundred and forty nine thousand eight hundred and eighty nine EURO and forty five eurocents), corresponding to 5% of the maximum grant amount (see Article 5.1), is retained by the Commission from the pre-financing payment and transferred into the '**Guarantee Fund**'.

### 21.3 Interim payments — Amount — Calculation

Interim payments reimburse the eligible costs incurred for the implementation of the action during the corresponding reporting periods.

The Commission will pay to the coordinator the amount due as interim payment within 90 days from receiving the periodic report (see Article 20.3), except if Articles 47 or 48 apply.

Payment is subject to the approval of the periodic report. Its approval does not imply recognition of the compliance, authenticity, completeness or correctness of its content.

The **amount due as interim payment** is calculated by the Commission in the following steps:

Step 1 — Application of the reimbursement rates

Step 2 — Limit to 90% of the maximum grant amount

### 21.3.1 Step 1 — Application of the reimbursement rates

The reimbursement rate(s) (see Article 5.2) are applied to the eligible costs (actual costs, unit costs and flat-rate costs; see Article 6) declared by the beneficiaries (see Article 20) and approved by the Commission (see above) for the concerned reporting period.

### 21.3.2 Step 2 — Limit to 90% of the maximum grant amount

The total amount of pre-financing and interim payments must not exceed 90% of the maximum grant amount set out in Article 5.1. The maximum amount for the interim payment will be calculated as follows:

{90% of the maximum grant amount (see Article 5.1)

minus

{pre-financing and previous interim payments}}.

## 21.4 Payment of the balance — Amount — Calculation — Release of the amount retained for the Guarantee Fund

The payment of the balance reimburses the remaining part of the eligible costs incurred by the beneficiaries for the implementation of the action.

If the total amount of earlier payments is greater than the final grant amount (see Article 5.3), the payment of the balance takes the form of a recovery (see Article 44).

If the total amount of earlier payments is lower than the final grant amount, the Commission will pay the balance within 90 days from receiving the final report (see Article 20.4), except if Articles 47 or 48 apply.

Payment is subject to the approval of the final report. Its approval does not imply recognition of the compliance, authenticity, completeness or correctness of its content.

The **amount due as the balance** is calculated by the Commission by deducting the total amount of pre-financing and interim payments (if any) already made, from the final grant amount determined in accordance with Article 5.3:

{final grant amount (see Article 5.3)

minus

{pre-financing and interim payments (if any) made}}.

At the payment of the balance, the amount retained for the Guarantee Fund (see above) will be released and:

- if the balance is positive: the amount released will be paid in full to the coordinator together with the amount due as the balance;
- if the balance is negative (payment of the balance taking the form of recovery): it will be deducted from the amount released (see Article 44.1.2). If the resulting amount:
  - is positive, it will be paid to the coordinator
  - is negative, it will be recovered.

The amount to be paid may however be offset — without the beneficiaries' consent — against any other amount owed by a beneficiary to the Commission or an executive agency (under the EU or Euratom budget), up to the maximum EU contribution indicated, for that beneficiary, in the estimated budget (see Annex 2).

### **21.5 Notification of amounts due**

When making payments, the Commission will formally notify to the coordinator the amount due, specifying whether it concerns an interim payment or the payment of the balance.

For the payment of the balance, the notification will also specify the final grant amount.

In the case of reduction of the grant or recovery of undue amounts, the notification will be preceded by the contradictory procedure set out in Articles 43 and 44.

### **21.6 Currency for payments**

The Commission will make all payments in euro.

### **21.7 Payments to the coordinator — Distribution to the beneficiaries**

Payments will be made to the coordinator.

Payments to the coordinator will discharge the Commission from its payment obligation.

The coordinator must distribute the payments between the beneficiaries without unjustified delay.

Pre-financing may however be distributed only:

- (a) if the minimum number of beneficiaries set out in the call for proposals has acceded to the Agreement (see Article 56) and
- (b) to beneficiaries that have acceded to the Agreement (see Article 56).

### **21.8 Bank account for payments**

All payments will be made to the following bank account:



Name of bank: BANK OF IRELAND

Full name of the account holder: ATHLONE INSTITUTE OF TECHNOLOGY

IBAN code: IE44BOFI90163414517852

## 21.9 Costs of payment transfers

The cost of the payment transfers is borne as follows:

- the Commission bears the cost of transfers charged by its bank;
- the beneficiary bears the cost of transfers charged by its bank;
- the party causing a repetition of a transfer bears all costs of the repeated transfer.

## 21.10 Date of payment

Payments by the Commission are considered to have been carried out on the date when they are debited to its account.

## 21.11 Consequences of non-compliance

21.11.1 If the Commission does not pay within the payment deadlines (see above), the beneficiaries are entitled to **late-payment interest** at the rate applied by the European Central Bank (ECB) for its main refinancing operations in euros ('reference rate'), plus three and a half points. The reference rate is the rate in force on the first day of the month in which the payment deadline expires, as published in the C series of the *Official Journal of the European Union*.

If the late-payment interest is lower than or equal to EUR 200, it will be paid to the coordinator only upon request submitted within two months of receiving the late payment.

Late-payment interest is not due if all beneficiaries are EU Member States (including regional and local government authorities or other public bodies acting on behalf of a Member State for the purpose of this Agreement).

Suspension of the payment deadline or payments (see Articles 47 and 48) will not be considered as late payment.

Late-payment interest covers the period running from the day following the due date for payment (see above), up to and including the date of payment.

Late-payment interest is not considered for the purposes of calculating the final grant amount.

21.11.2 If the coordinator breaches any of its obligations under this Article, the grant may be reduced (see Article 43) and the Agreement or the participation of the coordinator may be terminated (see Article 50).

Such breaches may also lead to any of the other measures described in Chapter 6.

## ARTICLE 22 — CHECKS, REVIEWS, AUDITS AND INVESTIGATIONS — EXTENSION OF FINDINGS

### 22.1 Checks, reviews and audits by the Commission



### 22.1.1 Right to carry out checks

The Commission will — during the implementation of the action or afterwards — check the proper implementation of the action and compliance with the obligations under the Agreement, including assessing deliverables and reports.

For this purpose the Commission may be assisted by external persons or bodies.

The Commission may also request additional information in accordance with Article 17. The Commission may request beneficiaries to provide such information to it directly.

Information provided must be accurate, precise and complete and in the format requested, including electronic format.

### 22.1.2 Right to carry out reviews

The Commission may — during the implementation of the action or afterwards — carry out reviews on the proper implementation of the action (including assessment of deliverables and reports), compliance with the obligations under the Agreement and continued scientific or technological relevance of the action.

Reviews may be started up to two years after the payment of the balance. They will be formally notified to the coordinator or beneficiary concerned and will be considered to have started on the date of the formal notification.

If the review is carried out on a third party (see Articles 10 to 16), the beneficiary concerned must inform the third party.

The Commission may carry out reviews directly (using its own staff) or indirectly (using external persons or bodies appointed to do so). It will inform the coordinator or beneficiary concerned of the identity of the external persons or bodies. They have the right to object to the appointment on grounds of commercial confidentiality.

The coordinator or beneficiary concerned must provide — within the deadline requested — any information and data in addition to deliverables and reports already submitted (including information on the use of resources). The Commission may request beneficiaries to provide such information to it directly.

The coordinator or beneficiary concerned may be requested to participate in meetings, including with external experts.

For **on-the-spot** reviews, the beneficiaries must allow access to their sites and premises, including to external persons or bodies, and must ensure that information requested is readily available.

Information provided must be accurate, precise and complete and in the format requested, including electronic format.

On the basis of the review findings, a '**review report**' will be drawn up.

The Commission will formally notify the review report to the coordinator or beneficiary concerned, which has 30 days to formally notify observations ('**contradictory review procedure**').

Reviews (including review reports) are in the language of the Agreement.

### 22.1.3 Right to carry out audits

The Commission may — during the implementation of the action or afterwards — carry out audits on the proper implementation of the action and compliance with the obligations under the Agreement.

Audits may be started up to two years after the payment of the balance. They will be formally notified to the coordinator or beneficiary concerned and will be considered to have started on the date of the formal notification.

If the audit is carried out on a third party (see Articles 10 to 16), the beneficiary concerned must inform the third party.

The Commission may carry out audits directly (using its own staff) or indirectly (using external persons or bodies appointed to do so). It will inform the coordinator or beneficiary concerned of the identity of the external persons or bodies. They have the right to object to the appointment on grounds of commercial confidentiality.

The coordinator or beneficiary concerned must provide — within the deadline requested — any information (including complete accounts, individual salary statements or other personal data) to verify compliance with the Agreement. The Commission may request beneficiaries to provide such information to it directly.

For **on-the-spot** audits, the beneficiaries must allow access to their sites and premises, including to external persons or bodies, and must ensure that information requested is readily available.

Information provided must be accurate, precise and complete and in the format requested, including electronic format.

On the basis of the audit findings, a '**draft audit report**' will be drawn up.

The Commission will formally notify the draft audit report to the coordinator or beneficiary concerned, which has 30 days to formally notify observations ('**contradictory audit procedure**'). This period may be extended by the Commission in justified cases.

The '**final audit report**' will take into account observations by the coordinator or beneficiary concerned. The report will be formally notified to it.

Audits (including audit reports) are in the language of the Agreement.

The Commission may also access the beneficiaries' statutory records for the periodical assessment of unit costs or flat-rate amounts.

## 22.2 Investigations by the European Anti-Fraud Office (OLAF)

Under Regulations No 883/2013<sup>16</sup> and No 2185/96<sup>17</sup> (and in accordance with their provisions and

<sup>16</sup> Regulation (EU, Euratom) No 883/2013 of the European Parliament and of the Council of 11 September 2013 concerning investigations conducted by the European Anti-Fraud Office (OLAF) and repealing Regulation (EC) No 1073/1999 of the European Parliament and of the Council and Council Regulation (Euratom) No 1074/1999 (OJ L 248, 18.09.2013, p. 1).

<sup>17</sup> Council Regulation (Euratom, EC) No 2185/1996 of 11 November 1996 concerning on-the-spot checks and inspections carried out by the Commission in order to protect the European Communities' financial interests against fraud and other irregularities (OJ L 292, 15.11.1996, p. 2).

procedures), the European Anti-Fraud Office (OLAF) may — at any moment during implementation of the action or afterwards — carry out investigations, including on-the-spot checks and inspections, to establish whether there has been fraud, corruption or any other illegal activity affecting the financial interests of the EU.

### 22.3 Checks and audits by the European Court of Auditors (ECA)

Under Article 287 of the Treaty on the Functioning of the European Union (TFEU) and Article 161 of the Financial Regulation No 966/2012<sup>18</sup>, the European Court of Auditors (ECA) may — at any moment during implementation of the action or afterwards — carry out audits.

The ECA has the right of access for the purpose of checks and audits.

### 22.4 Checks, reviews, audits and investigations for international organisations

Not applicable

### 22.5 Consequences of findings in checks, reviews, audits and investigations — Extension of findings

#### 22.5.1 Findings in this grant

Findings in checks, reviews, audits or investigations carried out in the context of this grant may lead to the rejection of ineligible costs (see Article 42), reduction of the grant (see Article 43), recovery of undue amounts (see Article 44) or to any of the other measures described in Chapter 6.

Rejection of costs or reduction of the grant after the payment of the balance will lead to a revised final grant amount (see Article 5.4).

Findings in checks, reviews, audits or investigations may lead to a request for amendment for the modification of Annex 1 (see Article 55).

Checks, reviews, audits or investigations that find systemic or recurrent errors, irregularities, fraud or breach of obligations may also lead to consequences in other EU or Euratom grants awarded under similar conditions (**‘extension of findings from this grant to other grants’**).

Moreover, findings arising from an OLAF investigation may lead to criminal prosecution under national law.

#### 22.5.2 Findings in other grants

The Commission may extend findings from other grants to this grant (**‘extension of findings from other grants to this grant’**), if:

- (a) the beneficiary concerned is found, in other EU or Euratom grants awarded under similar conditions, to have committed systemic or recurrent errors, irregularities, fraud or breach of obligations that have a material impact on this grant and

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<sup>18</sup> Regulation (EU, Euratom) No 966/2012 of the European Parliament and of the Council of 25 October 2012 on the financial rules applicable to the general budget of the Union and repealing Council Regulation (EC, Euratom) No 1605/2002 (OJ L 298, 26.10.2012, p. 1).

- (b) those findings are formally notified to the beneficiary concerned — together with the list of grants affected by the findings — no later than two years after the payment of the balance of this grant.

The extension of findings may lead to the rejection of costs (see Article 42), reduction of the grant (see Article 43), recovery of undue amounts (see Article 44), suspension of payments (see Article 48), suspension of the action implementation (see Article 49) or termination (see Article 50).

### 22.5.3 Procedure

The Commission will formally notify the beneficiary concerned the systemic or recurrent errors and its intention to extend these audit findings, together with the list of grants affected.

22.5.3.1 If the findings concern **eligibility of costs**: the formal notification will include:

- (a) an invitation to submit observations on the list of grants affected by the findings;
- (b) the request to submit **revised financial statements** for all grants affected;
- (c) the **correction rate for extrapolation** established by the Commission on the basis of the systemic or recurrent errors, to calculate the amounts to be rejected if the beneficiary concerned:
  - (i) considers that the submission of revised financial statements is not possible or practicable or
  - (ii) does not submit revised financial statements.

The beneficiary concerned has 90 days from receiving notification to submit observations, revised financial statements or to propose a duly substantiated **alternative correction method**. This period may be extended by the Commission in justified cases.

The Commission may then start a rejection procedure in accordance with Article 42, on the basis of:

- the revised financial statements, if approved;
  - the proposed alternative correction method, if accepted
- or
- the initially notified correction rate for extrapolation, if it does not receive any observations or revised financial statements, does not accept the observations or the proposed alternative correction method or does not approve the revised financial statements.

22.5.3.2 If the findings concern **substantial errors, irregularities or fraud or serious breach of obligations**: the formal notification will include:

- (a) an invitation to submit observations on the list of grants affected by the findings and
- (b) the flat-rate the Commission intends to apply according to the principle of proportionality.

The beneficiary concerned has 90 days from receiving notification to submit observations or to propose a duly substantiated alternative flat-rate.

The Commission may then start a reduction procedure in accordance with Article 43, on the basis of:

- the proposed alternative flat-rate, if accepted

or

- the initially notified flat-rate, if it does not receive any observations or does not accept the observations or the proposed alternative flat-rate.

## **22.6 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, any insufficiently substantiated costs will be ineligible (see Article 6) and will be rejected (see Article 42).

Such breaches may also lead to any of the other measures described in Chapter 6.

## **ARTICLE 23 — EVALUATION OF THE IMPACT OF THE ACTION**

### **23.1 Right to evaluate the impact of the action**

The Commission may carry out interim and final evaluations of the impact of the action measured against the objective of the EU programme.

Evaluations may be started during implementation of the action and up to five years after the payment of the balance. The evaluation is considered to start on the date of the formal notification to the coordinator or beneficiaries.

The Commission may make these evaluations directly (using its own staff) or indirectly (using external bodies or persons it has authorised to do so).

The coordinator or beneficiaries must provide any information relevant to evaluate the impact of the action, including information in electronic format.

### **23.2 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the Commission may apply the measures described in Chapter 6.

## **SECTION 3 RIGHTS AND OBLIGATIONS RELATED TO BACKGROUND AND RESULTS**

### **SUBSECTION 1 GENERAL**

#### **ARTICLE 23a — MANAGEMENT OF INTELLECTUAL PROPERTY**

##### **23a.1 Obligation to take measures to implement the Commission Recommendation on the management of intellectual property in knowledge transfer activities**

Beneficiaries that are universities or other public research organisations must take measures to

implement the principles set out in Points 1 and 2 of the Code of Practice annexed to the Commission Recommendation on the management of intellectual property in knowledge transfer activities<sup>19</sup>.

This does not change the obligations set out in Subsections 2 and 3 of this Section.

The beneficiaries must ensure that researchers and third parties involved in the action are aware of them.

### **23a.2 Consequences of non-compliance**

If a beneficiary breaches its obligations under this Article, the Commission may apply any of the measures described in Chapter 6.

## **SUBSECTION 2 RIGHTS AND OBLIGATIONS RELATED TO BACKGROUND**

### **ARTICLE 24 — AGREEMENT ON BACKGROUND**

#### **24.1 Agreement on background**

The beneficiaries must identify and agree (in writing) on the background for the action (**‘agreement on background’**).

**‘Background’** means any data, know-how or information — whatever its form or nature (tangible or intangible), including any rights such as intellectual property rights — that:

- (a) is held by the beneficiaries before they acceded to the Agreement, and
- (b) is needed to implement the action or exploit the results.

#### **24.2 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

### **ARTICLE 25 — ACCESS RIGHTS TO BACKGROUND**

#### **25.1 Exercise of access rights — Waiving of access rights — No sub-licensing**

To exercise access rights, this must first be requested in writing (**‘request for access’**).

**‘Access rights’** means rights to use results or background under the terms and conditions laid down in this Agreement.

Waivers of access rights are not valid unless in writing.

Unless agreed otherwise, access rights do not include the right to sub-license.

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<sup>19</sup> Commission Recommendation C(2008) 1329 of 10.4.2008 on the management of intellectual property in knowledge transfer activities and the Code of Practice for universities and other public research institutions attached to this recommendation.

## 25.2 Access rights for other beneficiaries, for implementing their own tasks under the action

The beneficiaries must give each other access — on a royalty-free basis — to background needed to implement their own tasks under the action, unless the beneficiary that holds the background has — before acceding to the Agreement —:

- (a) informed the other beneficiaries that access to its background is subject to legal restrictions or limits, including those imposed by the rights of third parties (including personnel), or
- (b) agreed with the other beneficiaries that access would not be on a royalty-free basis.

## 25.3 Access rights for other beneficiaries, for exploiting their own results

The beneficiaries must give each other access — under fair and reasonable conditions — to background needed for exploiting their own results, unless the beneficiary that holds the background has — before acceding to the Agreement — informed the other beneficiaries that access to its background is subject to legal restrictions or limits, including those imposed by the rights of third parties (including personnel).

‘**Fair and reasonable conditions**’ means appropriate conditions, including possible financial terms or royalty-free conditions, taking into account the specific circumstances of the request for access, for example the actual or potential value of the results or background to which access is requested and/or the scope, duration or other characteristics of the exploitation envisaged.

Requests for access may be made — unless agreed otherwise — up to one year after the period set out in Article 3.

## 25.4 Access rights for affiliated entities

Unless otherwise agreed in the consortium agreement, access to background must also be given — under fair and reasonable conditions (see above; Article 25.3) and unless it is subject to legal restrictions or limits, including those imposed by the rights of third parties (including personnel) — to affiliated entities<sup>20</sup> established in an EU Member State or ‘**associated country**’<sup>21</sup>, if this is needed to exploit the results generated by the beneficiaries to which they are affiliated.

<sup>20</sup> For the definition see Article 2.1(2) Rules for Participation Regulation No 1290/2013: ‘**affiliated entity**’ means any legal entity that is:

- under the direct or indirect control of a participant, or
- under the same direct or indirect control as the participant, or
- directly or indirectly controlling a participant.

‘Control’ may take any of the following forms:

- (a) the direct or indirect holding of more than 50% of the nominal value of the issued share capital in the legal entity concerned, or of a majority of the voting rights of the shareholders or associates of that entity;
- (b) the direct or indirect holding, in fact or in law, of decision-making powers in the legal entity concerned.

However the following relationships between legal entities shall not in themselves be deemed to constitute controlling relationships:

- (a) the same public investment corporation, institutional investor or venture-capital company has a direct or indirect holding of more than 50% of the nominal value of the issued share capital or a majority of voting rights of the shareholders or associates;
- (b) the legal entities concerned are owned or supervised by the same public body.

<sup>21</sup> For the definition, see Article 2.1(3) of the Rules for Participation Regulation No 1290/2013: ‘**associated country**’ means a third country which is party to an international agreement with the Union, as identified in Article 7 of Horizon 2020 Framework Programme Regulation No 1291/2013. Article 7 sets out the conditions for association of non-EU countries to Horizon 2020.



Unless agreed otherwise (see above; Article 25.1), the affiliated entity concerned must make the request directly to the beneficiary that holds the background.

Requests for access may be made — unless agreed otherwise — up to one year after the period set out in Article 3.

### **25.5 Access rights for third parties**

Not applicable

### **25.6 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

## **SUBSECTION 3 RIGHTS AND OBLIGATIONS RELATED TO RESULTS**

### **ARTICLE 26 — OWNERSHIP OF RESULTS**

#### **26.1 Ownership by the beneficiary that generates the results**

Results are owned by the beneficiary that generates them.

‘**Results**’ means any (tangible or intangible) output of the action such as data, knowledge or information — whatever its form or nature, whether it can be protected or not — that is generated in the action, as well as any rights attached to it, including intellectual property rights.

#### **26.2 Joint ownership by several beneficiaries**

Two or more beneficiaries own results jointly if:

- (a) they have jointly generated them and
- (b) it is not possible to:
  - (i) establish the respective contribution of each beneficiary, or
  - (ii) separate them for the purpose of applying for, obtaining or maintaining their protection (see Article 27).

The joint owners must agree (in writing) on the allocation and terms of exercise of their joint ownership (**‘joint ownership agreement’**), to ensure compliance with their obligations under this Agreement.

Unless otherwise agreed in the joint ownership agreement, each joint owner may grant non-exclusive licences to third parties to exploit jointly-owned results (without any right to sub-license), if the other joint owners are given:

- (a) at least 45 days advance notice and
- (b) fair and reasonable compensation.



Once the results have been generated, joint owners may agree (in writing) to apply another regime than joint ownership (such as, for instance, transfer to a single owner (see Article 30) with access rights for the others).

### **26.3 Rights of third parties (including personnel)**

If third parties (including personnel) may claim rights to the results, the beneficiary concerned must ensure that it complies with its obligations under the Agreement.

If a third party generates results, the beneficiary concerned must obtain all necessary rights (transfer, licences or other) from the third party, in order to be able to respect its obligations as if those results were generated by the beneficiary itself.

If obtaining the rights is impossible, the beneficiary must refrain from using the third party to generate the results.

### **26.4 EU ownership, to protect results**

26.4.1 The EU may — with the consent of the beneficiary concerned — assume ownership of results to protect them, if a beneficiary intends — up to four years after the period set out in Article 3 — to disseminate its results without protecting them, except in any of the following cases:

- (a) the lack of protection is because protecting the results is not possible, reasonable or justified (given the circumstances);
- (b) the lack of protection is because there is a lack of potential for commercial or industrial exploitation, or
- (c) the beneficiary intends to transfer the results to another beneficiary or third party established in an EU Member State or associated country, which will protect them.

Before the results are disseminated and unless any of the cases above under Points (a), (b) or (c) applies, the beneficiary must formally notify the Commission and at the same time inform it of any reasons for refusing consent. The beneficiary may refuse consent only if it can show that its legitimate interests would suffer significant harm.

If the Commission decides to assume ownership, it will formally notify the beneficiary concerned within 45 days of receiving notification.

No dissemination relating to these results may take place before the end of this period or, if the Commission takes a positive decision, until it has taken the necessary steps to protect the results.

26.4.2 The EU may — with the consent of the beneficiary concerned — assume ownership of results to protect them, if a beneficiary intends — up to four years after the period set out in Article 3 — to stop protecting them or not to seek an extension of protection, except in any of the following cases:

- (a) the protection is stopped because of a lack of potential for commercial or industrial exploitation;
- (b) an extension would not be justified given the circumstances.

A beneficiary that intends to stop protecting results or not seek an extension must — unless any of the cases above under Points (a) or (b) applies — formally notify the Commission at least 60 days

before the protection lapses or its extension is no longer possible and at the same time inform it of any reasons for refusing consent. The beneficiary may refuse consent only if it can show that its legitimate interests would suffer significant harm.

If the Commission decides to assume ownership, it will formally notify the beneficiary concerned within 45 days of receiving notification.

## **26.5 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to the any of the other measures described in Chapter 6.

## **ARTICLE 27 — PROTECTION OF RESULTS — VISIBILITY OF EU FUNDING**

### **27.1 Obligation to protect the results**

Each beneficiary must examine the possibility of protecting its results and must adequately protect them — for an appropriate period and with appropriate territorial coverage — if:

- (a) the results can reasonably be expected to be commercially or industrially exploited and
- (b) protecting them is possible, reasonable and justified (given the circumstances).

When deciding on protection, the beneficiary must consider its own legitimate interests and the legitimate interests (especially commercial) of the other beneficiaries.

### **27.2 EU ownership, to protect the results**

If a beneficiary intends not to protect its results, to stop protecting them or not seek an extension of protection, the EU may — under certain conditions (see Article 26.4) — assume ownership to ensure their (continued) protection.

### **27.3 Information on EU funding**

Applications for protection of results (including patent applications) filed by or on behalf of a beneficiary must — unless the Commission requests or agrees otherwise or unless it is impossible — include the following:

“The project leading to this application has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 870292”.

### **27.4 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such a breach may also lead to any of the other measures described in Chapter 6.

## **ARTICLE 28 — EXPLOITATION OF RESULTS**

## 28.1 Obligation to exploit the results

Each beneficiary must — up to four years after the period set out in Article 3 — take measures aiming to ensure ‘**exploitation**’ of its results (either directly or indirectly, in particular through transfer or licensing; see Article 30) by:

- (a) using them in further research activities (outside the action);
- (b) developing, creating or marketing a product or process;
- (c) creating and providing a service, or
- (d) using them in standardisation activities.

This does not change the security obligations in Article 37, which still apply.

## 28.2 Results that could contribute to European or international standards — Information on EU funding

If results are incorporated in a standard, the beneficiary concerned must — unless the Commission requests or agrees otherwise or unless it is impossible — ask the standardisation body to include the following statement in (information related to) the standard:

“Results incorporated in this standard received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 870292”.

## 28.3 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced in accordance with Article 43.

Such a breach may also lead to any of the other measures described in Chapter 6.

# ARTICLE 29 — DISSEMINATION OF RESULTS — OPEN ACCESS — VISIBILITY OF EU FUNDING

## 29.1 Obligation to disseminate results

Unless it goes against their legitimate interests, each beneficiary must — as soon as possible — ‘**disseminate**’ its results by disclosing them to the public by appropriate means (other than those resulting from protecting or exploiting the results), including in scientific publications (in any medium).

This does not change the obligation to protect results in Article 27, the confidentiality obligations in Article 36, the security obligations in Article 37 or the obligations to protect personal data in Article 39, all of which still apply.

A beneficiary that intends to disseminate its results must give advance notice to the other beneficiaries of — unless agreed otherwise — at least 45 days, together with sufficient information on the results it will disseminate.

Any other beneficiary may object within — unless agreed otherwise — 30 days of receiving

notification, if it can show that its legitimate interests in relation to the results or background would be significantly harmed. In such cases, the dissemination may not take place unless appropriate steps are taken to safeguard these legitimate interests.

If a beneficiary intends not to protect its results, it may — under certain conditions (see Article 26.4.1) — need to formally notify the Commission before dissemination takes place.

## 29.2 Open access to scientific publications

Each beneficiary must ensure open access (free of charge online access for any user) to all peer-reviewed scientific publications relating to its results.

In particular, it must:

- (a) as soon as possible and at the latest on publication, deposit a machine-readable electronic copy of the published version or final peer-reviewed manuscript accepted for publication in a repository for scientific publications;

Moreover, the beneficiary must aim to deposit at the same time the research data needed to validate the results presented in the deposited scientific publications.

- (b) ensure open access to the deposited publication — via the repository — at the latest:
  - (i) on publication, if an electronic version is available for free via the publisher, or
  - (ii) within six months of publication (twelve months for publications in the social sciences and humanities) in any other case.
- (c) ensure open access — via the repository — to the bibliographic metadata that identify the deposited publication.

The bibliographic metadata must be in a standard format and must include all of the following:

- the terms “European Union (EU)” and “Horizon 2020”;
- the name of the action, acronym and grant number;
- the publication date, and length of embargo period if applicable, and
- a persistent identifier.

## 29.3 Open access to research data

Regarding the digital research data generated in the action (**‘data’**), the beneficiaries must:

- (a) deposit in a research data repository and take measures to make it possible for third parties to access, mine, exploit, reproduce and disseminate — free of charge for any user — the following:
  - (i) the data, including associated metadata, needed to validate the results presented in scientific publications, as soon as possible;
  - (ii) not applicable;

- (iii) other data, including associated metadata, as specified and within the deadlines laid down in the ‘data management plan’ (see Annex 1);
- (b) provide information — via the repository — about tools and instruments at the disposal of the beneficiaries and necessary for validating the results (and — where possible — provide the tools and instruments themselves).

This does not change the obligation to protect results in Article 27, the confidentiality obligations in Article 36, the security obligations in Article 37 or the obligations to protect personal data in Article 39, all of which still apply.

As an exception, the beneficiaries do not have to ensure open access to specific parts of their research data under Point (a)(i) and (iii), if the achievement of the action's main objective (as described in Annex 1) would be jeopardised by making those specific parts of the research data openly accessible. In this case, the data management plan must contain the reasons for not giving access.

#### **29.4 Information on EU funding — Obligation and right to use the EU emblem**

Unless the Commission requests or agrees otherwise or unless it is impossible, any dissemination of results (in any form, including electronic) must:

- (a) display the EU emblem and
- (b) include the following text:

“This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 870292”.

When displayed together with another logo, the EU emblem must have appropriate prominence.

For the purposes of their obligations under this Article, the beneficiaries may use the EU emblem without first obtaining approval from the Commission.

This does not however give them the right to exclusive use.

Moreover, they may not appropriate the EU emblem or any similar trademark or logo, either by registration or by any other means.

#### **29.5 Disclaimer excluding Commission responsibility**

Any dissemination of results must indicate that it reflects only the author's view and that the Commission is not responsible for any use that may be made of the information it contains.

#### **29.6 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such a breach may also lead to any of the other measures described in Chapter 6.

### **ARTICLE 30 — TRANSFER AND LICENSING OF RESULTS**

### **30.1 Transfer of ownership**

Each beneficiary may transfer ownership of its results.

It must however ensure that its obligations under Articles 26.2, 26.4, 27, 28, 29, 30 and 31 also apply to the new owner and that this owner has the obligation to pass them on in any subsequent transfer.

This does not change the security obligations in Article 37, which still apply.

Unless agreed otherwise (in writing) for specifically-identified third parties or unless impossible under applicable EU and national laws on mergers and acquisitions, a beneficiary that intends to transfer ownership of results must give at least 45 days advance notice (or less if agreed in writing) to the other beneficiaries that still have (or still may request) access rights to the results. This notification must include sufficient information on the new owner to enable any beneficiary concerned to assess the effects on its access rights.

Unless agreed otherwise (in writing) for specifically-identified third parties, any other beneficiary may object within 30 days of receiving notification (or less if agreed in writing), if it can show that the transfer would adversely affect its access rights. In this case, the transfer may not take place until agreement has been reached between the beneficiaries concerned.

### **30.2 Granting licenses**

Each beneficiary may grant licences to its results (or otherwise give the right to exploit them), if:

- (a) this does not impede the access rights under Article 31 and
- (b) not applicable.

In addition to Points (a) and (b), exclusive licences for results may be granted only if all the other beneficiaries concerned have waived their access rights (see Article 31.1).

This does not change the dissemination obligations in Article 29 or security obligations in Article 37, which still apply.

### **30.3 Commission right to object to transfers or licensing**

Not applicable

### **30.4 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such a breach may also lead to any of the other measures described in Chapter 6.

## **ARTICLE 31 — ACCESS RIGHTS TO RESULTS**

### **31.1 Exercise of access rights — Waiving of access rights — No sub-licensing**

The conditions set out in Article 25.1 apply.

The obligations set out in this Article do not change the security obligations in Article 37, which still apply.

### **31.2 Access rights for other beneficiaries, for implementing their own tasks under the action**

The beneficiaries must give each other access — on a royalty-free basis — to results needed for implementing their own tasks under the action.

### **31.3 Access rights for other beneficiaries, for exploiting their own results**

The beneficiaries must give each other — under fair and reasonable conditions (see Article 25.3) — access to results needed for exploiting their own results.

Requests for access may be made — unless agreed otherwise — up to one year after the period set out in Article 3.

### **31.4 Access rights of affiliated entities**

Unless agreed otherwise in the consortium agreement, access to results must also be given — under fair and reasonable conditions (Article 25.3) — to affiliated entities established in an EU Member State or associated country, if this is needed for those entities to exploit the results generated by the beneficiaries to which they are affiliated.

Unless agreed otherwise (see above; Article 31.1), the affiliated entity concerned must make any such request directly to the beneficiary that owns the results.

Requests for access may be made — unless agreed otherwise — up to one year after the period set out in Article 3.

### **31.5 Access rights for the EU institutions, bodies, offices or agencies and EU Member States**

The beneficiaries must give access to their results — on a royalty-free basis — to EU institutions, bodies, offices or agencies, for developing, implementing or monitoring EU policies or programmes.

Such access rights are limited to non-commercial and non-competitive use.

This does not change the right to use any material, document or information received from the beneficiaries for communication and publicising activities (see Article 38.2).

### **31.6 Access rights for third parties**

Not applicable

### **31.7 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

## **SECTION 4 OTHER RIGHTS AND OBLIGATIONS**

## **ARTICLE 32 — RECRUITMENT AND WORKING CONDITIONS FOR RESEARCHERS**

### **32.1 Obligation to take measures to implement the European Charter for Researchers and Code of Conduct for the Recruitment of Researchers**

The beneficiaries must take all measures to implement the principles set out in the Commission Recommendation on the European Charter for Researchers and the Code of Conduct for the Recruitment of Researchers<sup>23</sup>, in particular regarding:

- working conditions;
- transparent recruitment processes based on merit, and
- career development.

The beneficiaries must ensure that researchers and third parties involved in the action are aware of them.

### **32.2 Consequences of non-compliance**

If a beneficiary breaches its obligations under this Article, the Commission may apply any of the measures described in Chapter 6.

## **ARTICLE 33 — GENDER EQUALITY**

### **33.1 Obligation to aim for gender equality**

The beneficiaries must take all measures to promote equal opportunities between men and women in the implementation of the action. They must aim, to the extent possible, for a gender balance at all levels of personnel assigned to the action, including at supervisory and managerial level.

### **33.2 Consequences of non-compliance**

If a beneficiary breaches its obligations under this Article, the Commission may apply any of the measures described in Chapter 6.

## **ARTICLE 34 — ETHICS AND RESEARCH INTEGRITY**

### **34.1 Obligation to comply with ethical and research integrity principles**

The beneficiaries must carry out the action in compliance with:

- (a) ethical principles (including the highest standards of research integrity)
- and
- (b) applicable international, EU and national law.

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<sup>23</sup> Commission Recommendation 2005/251/EC of 11 March 2005 on the European Charter for Researchers and on a Code of Conduct for the Recruitment of Researchers (OJ L 75, 22.3.2005, p. 67).



Funding will not be granted for activities carried out outside the EU if they are prohibited in all Member States or for activities which destroy human embryos (for example, for obtaining stem cells).

The beneficiaries must ensure that the activities under the action have an exclusive focus on civil applications.

The beneficiaries must ensure that the activities under the action do not:

- (a) aim at human cloning for reproductive purposes;
- (b) intend to modify the genetic heritage of human beings which could make such changes heritable (with the exception of research relating to cancer treatment of the gonads, which may be financed), or
- (c) intend to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer.

In addition, the beneficiaries must respect the fundamental principle of research integrity — as set out, for instance, in the European Code of Conduct for Research Integrity<sup>24</sup>.

This implies compliance with the following fundamental principles:

- **reliability** in ensuring the quality of research reflected in the design, the methodology, the analysis and the use of resources;
- **honesty** in developing, undertaking, reviewing, reporting and communicating research in a transparent, fair and unbiased way;
- **respect** for colleagues, research participants, society, ecosystems, cultural heritage and the environment;
- **accountability** for the research from idea to publication, for its management and organisation, for training, supervision and mentoring, and for its wider impacts

and means that beneficiaries must ensure that persons carrying out research tasks follow the good research practices and refrain from the research integrity violations described in this Code.

This does not change the other obligations under this Agreement or obligations under applicable international, EU or national law, all of which still apply.

### 34.2 Activities raising ethical issues

Activities raising ethical issues must comply with the ‘**ethics requirements**’ set out as deliverables in Annex 1.

Before the beginning of an activity raising an ethical issue, each beneficiary must have obtained:

- (a) any ethics committee opinion required under national law and

<sup>24</sup> European Code of Conduct for Research Integrity of ALLEA (All European Academies)  
[http://ec.europa.eu/research/participants/data/ref/h2020/other/hi/h2020-ethics\\_code-of-conduct\\_en.pdf](http://ec.europa.eu/research/participants/data/ref/h2020/other/hi/h2020-ethics_code-of-conduct_en.pdf)

- (b) any notification or authorisation for activities raising ethical issues required under national and/or European law

needed for implementing the action tasks in question.

The documents must be kept on file and be submitted upon request by the coordinator to the Commission (see Article 52). If they are not in English, they must be submitted together with an English summary, which shows that the action tasks in question are covered and includes the conclusions of the committee or authority concerned (if available).

### **34.3 Activities involving human embryos or human embryonic stem cells**

Activities involving research on human embryos or human embryonic stem cells may be carried out, in addition to Article 34.1, only if:

- they are set out in Annex 1 or
- the coordinator has obtained explicit approval (in writing) from the Commission (see Article 52).

### **34.4 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43) and the Agreement or participation of the beneficiary may be terminated (see Article 50).

Such breaches may also lead to any of the other measures described in Chapter 6.

## **ARTICLE 35 — CONFLICT OF INTERESTS**

### **35.1 Obligation to avoid a conflict of interests**

The beneficiaries must take all measures to prevent any situation where the impartial and objective implementation of the action is compromised for reasons involving economic interest, political or national affinity, family or emotional ties or any other shared interest (**‘conflict of interests’**).

They must formally notify to the Commission without delay any situation constituting or likely to lead to a conflict of interests and immediately take all the necessary steps to rectify this situation.

The Commission may verify that the measures taken are appropriate and may require additional measures to be taken by a specified deadline.

### **35.2 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43) and the Agreement or participation of the beneficiary may be terminated (see Article 50).

Such breaches may also lead to any of the other measures described in Chapter 6.

## **ARTICLE 36 — CONFIDENTIALITY**

### **36.1 General obligation to maintain confidentiality**

During implementation of the action and for four years after the period set out in Article 3, the parties must keep confidential any data, documents or other material (in any form) that is identified as confidential at the time it is disclosed (**‘confidential information’**).

If a beneficiary requests, the Commission may agree to keep such information confidential for an additional period beyond the initial four years.

If information has been identified as confidential only orally, it will be considered to be confidential only if this is confirmed in writing within 15 days of the oral disclosure.

Unless otherwise agreed between the parties, they may use confidential information only to implement the Agreement.

The beneficiaries may disclose confidential information to their personnel or third parties involved in the action only if they:

- (a) need to know to implement the Agreement and
- (b) are bound by an obligation of confidentiality.

This does not change the security obligations in Article 37, which still apply.

The Commission may disclose confidential information to its staff, other EU institutions and bodies. It may disclose confidential information to third parties, if:

- (a) this is necessary to implement the Agreement or safeguard the EU's financial interests and
- (b) the recipients of the information are bound by an obligation of confidentiality.

Under the conditions set out in Article 4 of the Rules for Participation Regulation No 1290/2013<sup>25</sup>, the Commission must moreover make available information on the results to other EU institutions, bodies, offices or agencies as well as Member States or associated countries.

The confidentiality obligations no longer apply if:

- (a) the disclosing party agrees to release the other party;
- (b) the information was already known by the recipient or is given to him without obligation of confidentiality by a third party that was not bound by any obligation of confidentiality;
- (c) the recipient proves that the information was developed without the use of confidential information;
- (d) the information becomes generally and publicly available, without breaching any confidentiality obligation, or
- (e) the disclosure of the information is required by EU or national law.

## 36.2 Consequences of non-compliance

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<sup>25</sup> Regulation (EU) No 1290/2013 of the European Parliament and of the Council of 11 December 2013 laying down the rules for participation and dissemination in "Horizon 2020 - the Framework Programme for Research and Innovation (2014-2020)" (OJ L 347, 20.12.2013 p.81).

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

## **ARTICLE 37 — SECURITY-RELATED OBLIGATIONS**

### **37.1 Results with a security recommendation**

Not applicable

### **37.2 Classified information**

Not applicable

### **37.3 Activities involving dual-use goods or dangerous materials and substances**

Not applicable

### **37.4 Consequences of non-compliance**

Not applicable

## **ARTICLE 38 — PROMOTING THE ACTION — VISIBILITY OF EU FUNDING**

### **38.1 Communication activities by beneficiaries**

#### **38.1.1 Obligation to promote the action and its results**

The beneficiaries must promote the action and its results, by providing targeted information to multiple audiences (including the media and the public) in a strategic and effective manner.

This does not change the dissemination obligations in Article 29, the confidentiality obligations in Article 36 or the security obligations in Article 37, all of which still apply.

Before engaging in a communication activity expected to have a major media impact, the beneficiaries must inform the Commission (see Article 52).

#### **38.1.2 Information on EU funding — Obligation and right to use the EU emblem**

Unless the Commission requests or agrees otherwise or unless it is impossible, any communication activity related to the action (including in electronic form, via social media, etc.) and any infrastructure, equipment and major results funded by the grant must:

- (a) display the EU emblem and
- (b) include the following text:

For communication activities:

“This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 870292”.

For infrastructure, equipment and major results:

“This *[infrastructure]/[equipment]/[insert type of result]* is part of a project that has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 870292”.

When displayed together with another logo, the EU emblem must have appropriate prominence.

For the purposes of their obligations under this Article, the beneficiaries may use the EU emblem without first obtaining approval from the Commission.

This does not, however, give them the right to exclusive use.

Moreover, they may not appropriate the EU emblem or any similar trademark or logo, either by registration or by any other means.

### 38.1.3 Disclaimer excluding Commission responsibility

Any communication activity related to the action must indicate that it reflects only the author's view and that the Commission is not responsible for any use that may be made of the information it contains.

## 38.2 Communication activities by the Commission

### 38.2.1 Right to use beneficiaries’ materials, documents or information

The Commission may use, for its communication and publicising activities, information relating to the action, documents notably summaries for publication and public deliverables as well as any other material, such as pictures or audio-visual material received from any beneficiary (including in electronic form).

This does not change the confidentiality obligations in Article 36 and the security obligations in Article 37, all of which still apply.

If the Commission’s use of these materials, documents or information would risk compromising legitimate interests, the beneficiary concerned may request the Commission not to use it (see Article 52).

The right to use a beneficiary’s materials, documents and information includes:

- (a) **use for its own purposes** (in particular, making them available to persons working for the Commission or any other EU institution, body, office or agency or body or institutions in EU Member States; and copying or reproducing them in whole or in part, in unlimited numbers);
- (b) **distribution to the public** (in particular, publication as hard copies and in electronic or digital format, publication on the internet, as a downloadable or non-downloadable file, broadcasting by any channel, public display or presentation, communicating through press information services, or inclusion in widely accessible databases or indexes);
- (c) **editing or redrafting** for communication and publicising activities (including shortening, summarising, inserting other elements (such as meta-data, legends, other graphic, visual, audio or text elements), extracting parts (e.g. audio or video files), dividing into parts, use in a compilation);

- (d) translation;
- (e) giving **access in response to individual requests** under Regulation No 1049/2001<sup>27</sup>, without the right to reproduce or exploit;
- (f) **storage** in paper, electronic or other form;
- (g) **archiving**, in line with applicable document-management rules, and
- (h) the right to authorise **third parties** to act on its behalf or sub-license the modes of use set out in Points (b), (c), (d) and (f) to third parties if needed for the communication and publicising activities of the Commission.

If the right of use is subject to rights of a third party (including personnel of the beneficiary), the beneficiary must ensure that it complies with its obligations under this Agreement (in particular, by obtaining the necessary approval from the third parties concerned).

Where applicable (and if provided by the beneficiaries), the Commission will insert the following information:

“© – [year] – [name of the copyright owner]. All rights reserved. Licensed to the European Union (EU) under conditions.”

### 38.3 Consequences of non-compliance

If a beneficiary breaches any of its obligations under this Article, the grant may be reduced (see Article 43).

Such breaches may also lead to any of the other measures described in Chapter 6.

## ARTICLE 39 — PROCESSING OF PERSONAL DATA

### 39.1 Processing of personal data by the Commission

Any personal data under the Agreement will be processed by the Commission under Regulation No 45/2001<sup>28</sup> and according to the ‘notifications of the processing operations’ to the Data Protection Officer (DPO) of the Commission (publicly accessible in the DPO register).

Such data will be processed by the ‘**data controller**’ of the Commission for the purposes of implementing, managing and monitoring the Agreement or protecting the financial interests of the EU or Euratom (including checks, reviews, audits and investigations; see Article 22).

The persons whose personal data are processed have the right to access and correct their own personal data. For this purpose, they must send any queries about the processing of their personal data to the data controller, via the contact point indicated in the privacy statement(s) that are published on the Commission websites.

<sup>27</sup> Regulation (EC) No 1049/2001 of the European Parliament and of the Council of 30 May 2001 regarding public access to European Parliament, Council and Commission documents, OJ L 145, 31.5.2001, p. 43.

<sup>28</sup> Regulation (EC) No 45/2001 of the European Parliament and of the Council of 18 December 2000 on the protection of individuals with regard to the processing of personal data by the Community institutions and bodies and on the free movement of such data (OJ L 8, 12.01.2001, p. 1).

They also have the right to have recourse at any time to the European Data Protection Supervisor (EDPS).

### **39.2 Processing of personal data by the beneficiaries**

The beneficiaries must process personal data under the Agreement in compliance with applicable EU and national law on data protection (including authorisations or notification requirements).

The beneficiaries may grant their personnel access only to data that is strictly necessary for implementing, managing and monitoring the Agreement.

The beneficiaries must inform the personnel whose personal data are collected and processed by the Commission. For this purpose, they must provide them with the privacy statement(s) (see above), before transmitting their data to the Commission.

### **39.3 Consequences of non-compliance**

If a beneficiary breaches any of its obligations under Article 39.2, the Commission may apply any of the measures described in Chapter 6.

## **ARTICLE 40 — ASSIGNMENTS OF CLAIMS FOR PAYMENT AGAINST THE COMMISSION**

The beneficiaries may not assign any of their claims for payment against the Commission to any third party, except if approved by the Commission on the basis of a reasoned, written request by the coordinator (on behalf of the beneficiary concerned).

If the Commission has not accepted the assignment or the terms of it are not observed, the assignment will have no effect on it.

In no circumstances will an assignment release the beneficiaries from their obligations towards the Commission.

## **CHAPTER 5 DIVISION OF BENEFICIARIES' ROLES AND RESPONSIBILITIES** **— RELATIONSHIP WITH COMPLEMENTARY BENEFICIARIES —** **RELATIONSHIP WITH PARTNERS OF A JOINT ACTION**

### **ARTICLE 41 — DIVISION OF BENEFICIARIES' ROLES AND RESPONSIBILITIES** **— RELATIONSHIP WITH COMPLEMENTARY BENEFICIARIES —** **RELATIONSHIP WITH PARTNERS OF A JOINT ACTION**

#### **41.1 Roles and responsibility towards the Commission**

The beneficiaries have full responsibility for implementing the action and complying with the Agreement.

The beneficiaries are jointly and severally liable for the **technical implementation** of the action as described in Annex 1. If a beneficiary fails to implement its part of the action, the other beneficiaries become responsible for implementing this part (without being entitled to any additional EU funding for doing so), unless the Commission expressly relieves them of this obligation.



The **financial responsibility** of each beneficiary is governed by Article 44.

## 41.2 Internal division of roles and responsibilities

The internal roles and responsibilities of the beneficiaries are divided as follows:

(a) Each **beneficiary** must:

- (i) keep information stored in the Participant Portal Beneficiary Register (via the electronic exchange system) up to date (see Article 17);
- (ii) inform the coordinator immediately of any events or circumstances likely to affect significantly or delay the implementation of the action (see Article 17);
- (iii) submit to the coordinator in good time:
  - individual financial statements for itself and, if required, certificates on the financial statements (see Article 20);
  - the data needed to draw up the technical reports (see Article 20);
  - ethics committee opinions and notifications or authorisations for activities raising ethical issues (see Article 34);
  - any other documents or information required by the Commission under the Agreement, unless the Agreement requires the beneficiary to submit this information directly to the Commission.

(b) The **coordinator** must:

- (i) monitor that the action is implemented properly (see Article 7);
- (ii) act as the intermediary for all communications between the beneficiaries and the Commission (in particular, providing the Commission with the information described in Article 17), unless the Agreement specifies otherwise;
- (iii) request and review any documents or information required by the Commission and verify their completeness and correctness before passing them on to the Commission;
- (iv) submit the deliverables and reports to the Commission (see Articles 19 and 20);
- (v) ensure that all payments are made to the other beneficiaries without unjustified delay (see Article 21);
- (vi) inform the Commission of the amounts paid to each beneficiary, when required under the Agreement (see Articles 44 and 50) or requested by the Commission.

The coordinator may not delegate or subcontract the above-mentioned tasks to any other beneficiary or third party (including linked third parties).

## 41.3 Internal arrangements between beneficiaries — Consortium agreement

The beneficiaries must have internal arrangements regarding their operation and co-ordination to



ensure that the action is implemented properly. These internal arrangements must be set out in a written ‘**consortium agreement**’ between the beneficiaries, which may cover:

- internal organisation of the consortium;
- management of access to the electronic exchange system;
- distribution of EU funding;
- additional rules on rights and obligations related to background and results (including whether access rights remain or not, if a beneficiary is in breach of its obligations) (see Section 3 of Chapter 4);
- settlement of internal disputes;
- liability, indemnification and confidentiality arrangements between the beneficiaries.

The consortium agreement must not contain any provision contrary to the Agreement.

#### **41.4 Relationship with complementary beneficiaries — Collaboration agreement**

Not applicable

#### **41.5 Relationship with partners of a joint action — Coordination agreement**

Not applicable

### **CHAPTER 6 REJECTION OF COSTS — REDUCTION OF THE GRANT — RECOVERY — SANCTIONS — DAMAGES — SUSPENSION — TERMINATION — FORCE MAJEURE**

#### **SECTION 1 REJECTION OF COSTS — REDUCTION OF THE GRANT — RECOVERY — SANCTIONS**

#### **ARTICLE 42 — REJECTION OF INELIGIBLE COSTS**

##### **42.1 Conditions**

The Commission will — after **termination of the participation of a beneficiary**, at the time of an **interim payment, at the payment of the balance or afterwards** — reject any costs which are ineligible (see Article 6), in particular following checks, reviews, audits or investigations (see Article 22).

The rejection may also be based on the **extension of findings from other grants to this grant** (see Article 22.5.2).

##### **42.2 Ineligible costs to be rejected — Calculation — Procedure**

Ineligible costs will be rejected in full.

If the rejection of costs does not lead to a recovery (see Article 44), the Commission will formally notify the coordinator or beneficiary concerned of the rejection of costs, the amounts and the reasons why (if applicable, together with the notification of amounts due; see Article 21.5). The coordinator or beneficiary concerned may — within 30 days of receiving notification — formally notify the Commission of its disagreement and the reasons why.

If the rejection of costs leads to a recovery, the Commission will follow the contradictory procedure with pre-information letter set out in Article 44.

### 42.3 Effects

If the Commission rejects costs at the time of an **interim payment or the payment of the balance**, it will deduct them from the total eligible costs declared, for the action, in the periodic or final summary financial statement (see Articles 20.3 and 20.4). It will then calculate the interim payment or payment of the balance as set out in Articles 21.3 or 21.4.

If the Commission rejects costs **after termination of the participation of a beneficiary**, it will deduct them from the costs declared by the beneficiary in the termination report and include the rejection in the calculation after termination (see Article 50.2 and 50.3).

If the Commission — **after an interim payment but before the payment of the balance** — rejects costs declared in a periodic summary financial statement, it will deduct them from the total eligible costs declared, for the action, in the next periodic summary financial statement or in the final summary financial statement. It will then calculate the interim payment or payment of the balance as set out in Articles 21.3 or 21.4.

If the Commission rejects costs **after the payment of the balance**, it will deduct the amount rejected from the total eligible costs declared, by the beneficiary, in the final summary financial statement. It will then calculate the revised final grant amount as set out in Article 5.4.

## ARTICLE 43 — REDUCTION OF THE GRANT

### 43.1 Conditions

The Commission may — **after termination of the participation of a beneficiary, at the payment of the balance or afterwards** — reduce the grant amount (see Article 5.1), if :

- (a) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed:
  - (i) substantial errors, irregularities or fraud or
  - (ii) serious breach of obligations under the Agreement or during the award procedure (including improper implementation of the action, submission of false information, failure to provide required information, breach of ethical principles) or
- (b) a beneficiary (or a natural person who has the power to represent or take decision on its behalf) has committed — in other EU or Euratom grants awarded to it under similar conditions — systemic or recurrent errors, irregularities, fraud or serious breach of obligations that have a material impact on this grant (**extension of findings from other grants to this grant**; see Article 22.5.2).

### 43.2 Amount to be reduced — Calculation — Procedure

The amount of the reduction will be proportionate to the seriousness of the errors, irregularities or fraud or breach of obligations.

Before reduction of the grant, the Commission will formally notify a '**pre-information letter**' to the coordinator or beneficiary concerned:

- informing it of its intention to reduce the grant, the amount it intends to reduce and the reasons why and
- inviting it to submit observations within 30 days of receiving notification.

If the Commission does not receive any observations or decides to pursue reduction despite the observations it has received, it will formally notify **confirmation** of the reduction (if applicable, together with the notification of amounts due; see Article 21).

### 43.3 Effects

If the Commission reduces the grant **after termination of the participation of a beneficiary**, it will calculate the reduced grant amount for that beneficiary and then determine the amount due to that beneficiary (see Article 50.2 and 50.3).

If the Commission reduces the grant **at the payment of the balance**, it will calculate the reduced grant amount for the action and then determine the amount due as payment of the balance (see Articles 5.3.4 and 21.4).

If the Commission reduces the grant **after the payment of the balance**, it will calculate the revised final grant amount for the beneficiary concerned (see Article 5.4). If the revised final grant amount for the beneficiary concerned is lower than its share of the final grant amount, the Commission will recover the difference (see Article 44).

## ARTICLE 44 — RECOVERY OF UNDUE AMOUNTS

### 44.1 Amount to be recovered — Calculation — Procedure

The Commission will — after **termination of the participation of a beneficiary, at the payment of the balance or afterwards** — claim back any amount that was paid, but is not due under the Agreement.

Each beneficiary's financial responsibility in case of recovery is limited to its own debt, except for the amount retained for the Guarantee Fund (see Article 21.4).

#### 44.1.1 Recovery after termination of a beneficiary's participation

If recovery takes place after termination of a beneficiary's participation (including the coordinator), the Commission will claim back the undue amount from the beneficiary concerned, by formally notifying it a debit note (see Article 50.2 and 50.3). This note will specify the amount to be recovered, the terms and the date for payment.

If payment is not made by the date specified in the debit note, the Commission will **recover** the amount:

- (a) by ‘**offsetting**’ it — without the beneficiary’s consent — against any amounts owed to the beneficiary concerned by the Commission or an executive agency (from the EU or Euratom budget).

In exceptional circumstances, to safeguard the EU’s financial interests, the Commission may offset before the payment date specified in the debit note;

- (b) not applicable;

- (c) by **taking legal action** (see Article 57) or by **adopting an enforceable decision** under Article 299 of the Treaty on the Functioning of the EU (TFEU) and Article 79(2) of the Financial regulation No 966/2012.

If payment is not made by the date specified in the debit note, the amount to be recovered (see above) will be increased by **late-payment interest** at the rate set out in Article 21.11, from the day following the payment date in the debit note, up to and including the date the Commission receives full payment of the amount.

Partial payments will be first credited against expenses, charges and late-payment interest and then against the principal.

Bank charges incurred in the recovery process will be borne by the beneficiary, unless Directive 2007/64/EC<sup>29</sup> applies.

#### 44.1.2 Recovery at payment of the balance

If the payment of the balance takes the form of a recovery (see Article 21.4), the Commission will formally notify a ‘**pre-information letter**’ to the coordinator:

- informing it of its intention to recover, the amount due as the balance and the reasons why;
- specifying that it intends to deduct the amount to be recovered from the amount retained for the Guarantee Fund;
- requesting the coordinator to submit a report on the distribution of payments to the beneficiaries within 30 days of receiving notification, and
- inviting the coordinator to submit observations within 30 days of receiving notification.

If no observations are submitted or the Commission decides to pursue recovery despite the observations it has received, it will **confirm recovery** (together with the notification of amounts due; see Article 21.5) and:

- pay the difference between the amount to be recovered and the amount retained for the Guarantee Fund, **if the difference is positive** or
- formally notify to the coordinator a **debit note** for the difference between the amount to be recovered and the amount retained for the Guarantee Fund, **if the difference is negative**. This note will also specify the terms and the date for payment.

<sup>29</sup> Directive 2007/64/EC of the European Parliament and of the Council of 13 November 2007 on payment services in the internal market amending Directives 97/7/EC, 2002/65/EC, 2005/60/EC and 2006/48/EC and repealing Directive 97/5/EC (OJ L 319, 05.12.2007, p. 1).

If the coordinator does not repay the Commission by the date in the debit note and has not submitted the report on the distribution of payments: the Commission will **recover** the amount set out in the debit note from the coordinator (see below).

If the coordinator does not repay the Commission by the date in the debit note, but has submitted the report on the distribution of payments: the Commission will:

- (a) identify the beneficiaries for which the amount calculated as follows is negative:

$\{ \{ \{ \text{beneficiary's costs declared in the final summary financial statement and approved by the Commission multiplied by the reimbursement rate set out in Article 5.2 for the beneficiary concerned} \}$

divided by

the EU contribution for the action calculated according to Article 5.3.1}

multiplied by

the final grant amount (see Article 5.3)},

minus

{pre-financing and interim payments received by the beneficiary} }.

- (b) formally notify to each beneficiary identified according to point (a) a **debit note** specifying the terms and date for payment. The amount of the debit note is calculated as follows:

$\{ \{ \text{amount calculated according to point (a) for the beneficiary concerned} \}$

divided by

the sum of the amounts calculated according to point (a) for all the beneficiaries identified according to point (a)}

multiplied by

the amount set out in the debit note formally notified to the coordinator}.

If payment is not made by the date specified in the debit note, the Commission will **recover** the amount:

- (a) by **offsetting** it — without the beneficiary's consent — against any amounts owed to the beneficiary concerned by the Commission or an executive agency (from the EU or Euratom budget).

In exceptional circumstances, to safeguard the EU's financial interests, the Commission may offset before the payment date specified in the debit note;

- (b) by **drawing on the Guarantee Fund**. The Commission will formally notify the beneficiary concerned the debit note on behalf of the Guarantee Fund and recover the amount:

- (i) not applicable;

- (ii) by **taking legal action** (see Article 57) or by **adopting an enforceable decision** under Article 299 of the Treaty on the Functioning of the EU (TFEU) and Article 79(2) of the Financial Regulation No 966/2012.

If payment is not made by the date in the debit note, the amount to be recovered (see above) will be increased by **late-payment interest** at the rate set out in Article 21.11, from the day following the payment date in the debit note, up to and including the date the Commission receives full payment of the amount.

Partial payments will be first credited against expenses, charges and late-payment interest and then against the principal.

Bank charges incurred in the recovery process will be borne by the beneficiary, unless Directive 2007/64/EC applies.

#### 44.1.3 Recovery of amounts after payment of the balance

If, for a beneficiary, the revised final grant amount (see Article 5.4) is lower than its share of the final grant amount, it must repay the difference to the Commission.

The beneficiary's share of the final grant amount is calculated as follows:

$$\left\{ \left\{ \text{beneficiary's costs declared in the final summary financial statement and approved by the Commission} \right. \right. \\ \left. \left. \text{multiplied by the reimbursement rate set out in Article 5.2 for the beneficiary concerned} \right\} \right. \\ \text{divided by} \\ \left. \left\{ \text{the EU contribution for the action calculated according to Article 5.3.1} \right\} \right. \\ \text{multiplied by} \\ \left. \left\{ \text{the final grant amount (see Article 5.3)} \right\} \right\}.$$

If the coordinator has not distributed amounts received (see Article 21.7), the Commission will also recover these amounts.

The Commission will formally notify a **pre-information letter** to the beneficiary concerned:

- informing it of its intention to recover, the due amount and the reasons why and
- inviting it to submit observations within 30 days of receiving notification.

If no observations are submitted or the Commission decides to pursue recovery despite the observations it has received, it will **confirm** the amount to be recovered and formally notify to the beneficiary concerned a **debit note**. This note will also specify the terms and the date for payment.

If payment is not made by the date specified in the debit note, the Commission will **recover** the amount:

- (a) by **offsetting** it — without the beneficiary's consent — against any amounts owed to the beneficiary concerned by the Commission or an executive agency (from the EU or Euratom budget).

In exceptional circumstances, to safeguard the EU's financial interests, the Commission may offset before the payment date specified in the debit note;

- (b) by **drawing on the Guarantee Fund**. The Commission will formally notify the beneficiary concerned the debit note on behalf of the Guarantee Fund and recover the amount:

- (i) not applicable;
- (ii) by **taking legal action** (see Article 57) or by **adopting an enforceable decision** under Article 299 of the Treaty on the Functioning of the EU (TFEU) and Article 79(2) of the Financial Regulation No 966/2012.

If payment is not made by the date in the debit note, the amount to be recovered (see above) will be increased by **late-payment interest** at the rate set out in Article 21.11, from the day following the date for payment in the debit note, up to and including the date the Commission receives full payment of the amount.

Partial payments will be first credited against expenses, charges and late-payment interest and then against the principal.

Bank charges incurred in the recovery process will be borne by the beneficiary, unless Directive 2007/64/EC applies.

## **ARTICLE 45 — ADMINISTRATIVE SANCTIONS**

In addition to contractual measures, the Commission may also adopt administrative sanctions under Articles 106 and 131(4) of the Financial Regulation No 966/2012 (i.e. exclusion from future procurement contracts, grants, prizes and expert contracts and/or financial penalties).

## **SECTION 2 LIABILITY FOR DAMAGES**

### **ARTICLE 46 — LIABILITY FOR DAMAGES**

#### **46.1 Liability of the Commission**

The Commission cannot be held liable for any damage caused to the beneficiaries or to third parties as a consequence of implementing the Agreement, including for gross negligence.

The Commission cannot be held liable for any damage caused by any of the beneficiaries or third parties involved in the action, as a consequence of implementing the Agreement.

#### **46.2 Liability of the beneficiaries**

Except in case of force majeure (see Article 51), the beneficiaries must compensate the Commission for any damage it sustains as a result of the implementation of the action or because the action was not implemented in full compliance with the Agreement.

## **SECTION 3 SUSPENSION AND TERMINATION**

### **ARTICLE 47 — SUSPENSION OF PAYMENT DEADLINE**

#### **47.1 Conditions**

The Commission may — at any moment — suspend the payment deadline (see Article 21.2 to 21.4) if a request for payment (see Article 20) cannot be approved because:



- (a) it does not comply with the provisions of the Agreement (see Article 20);
- (b) the technical or financial reports have not been submitted or are not complete or additional information is needed, or
- (c) there is doubt about the eligibility of the costs declared in the financial statements and additional checks, reviews, audits or investigations are necessary.

## 47.2 Procedure

The Commission will formally notify the coordinator of the suspension and the reasons why.

The suspension will **take effect** the day notification is sent by the Commission (see Article 52).

If the conditions for suspending the payment deadline are no longer met, the suspension will be **lifted** — and the remaining period will resume.

If the suspension exceeds two months, the coordinator may request the Commission if the suspension will continue.

If the payment deadline has been suspended due to the non-compliance of the technical or financial reports (see Article 20) and the revised report or statement is not submitted or was submitted but is also rejected, the Commission may also terminate the Agreement or the participation of the beneficiary (see Article 50.3.1(l)).

## ARTICLE 48 — SUSPENSION OF PAYMENTS

### 48.1 Conditions

The Commission may — at any moment — suspend payments, in whole or in part and interim payments or the payment of the balance for one or more beneficiaries, if:

- (a) a beneficiary (or a natural person who has the power to represent or take decision on its behalf) has committed or is suspected of having committed:
  - (i) substantial errors, irregularities or fraud or
  - (ii) serious breach of obligations under the Agreement or during the award procedure (including improper implementation of the action, submission of false information, failure to provide required information, breach of ethical principles) or
- (b) a beneficiary (or a natural person who has the power to represent or take decision on its behalf) has committed — in other EU or Euratom grants awarded to it under similar conditions — systemic or recurrent errors, irregularities, fraud or serious breach of obligations that have a material impact on this grant (**extension of findings from other grants to this grant**; see Article 22.5.2).

If payments are suspended for one or more beneficiaries, the Commission will make partial payment(s) for the part(s) not suspended. If suspension concerns the payment of the balance, — once suspension is lifted — the payment or the recovery of the amount(s) concerned will be considered the payment of the balance that closes the action.



## 48.2 Procedure

Before suspending payments, the Commission will formally notify the coordinator or beneficiary concerned:

- informing it of its intention to suspend payments and the reasons why and
- inviting it to submit observations within 30 days of receiving notification.

If the Commission does not receive observations or decides to pursue the procedure despite the observations it has received, it will formally notify **confirmation** of the suspension. Otherwise, it will formally notify that the suspension procedure is not continued.

The suspension will **take effect** the day the confirmation notification is sent by the Commission.

If the conditions for resuming payments are met, the suspension will be **lifted**. The Commission will formally notify the coordinator or beneficiary concerned.

During the suspension, the periodic report(s) for all reporting periods except the last one (see Article 20.3), must not contain any individual financial statements from the beneficiary concerned. The coordinator must include them in the next periodic report after the suspension is lifted or — if suspension is not lifted before the end of the action — in the last periodic report.

The beneficiaries may suspend implementation of the action (see Article 49.1) or terminate the Agreement or the participation of the beneficiary concerned (see Article 50.1 and 50.2).

## ARTICLE 49 — SUSPENSION OF THE ACTION IMPLEMENTATION

### 49.1 Suspension of the action implementation, by the beneficiaries

#### 49.1.1 Conditions

The beneficiaries may suspend implementation of the action or any part of it, if exceptional circumstances — in particular *force majeure* (see Article 51) — make implementation impossible or excessively difficult.

#### 49.1.2 Procedure

The coordinator must immediately formally notify to the Commission the suspension (see Article 52), stating:

- the reasons why and
- the expected date of resumption.

The suspension will **take effect** the day this notification is received by the Commission.

Once circumstances allow for implementation to resume, the coordinator must immediately formally notify the Commission and request an **amendment** of the Agreement to set the date on which the action will be resumed, extend the duration of the action and make other changes necessary to adapt the action to the new situation (see Article 55) — unless the Agreement or the participation of a beneficiary has been terminated (see Article 50).

The suspension will be **lifted** with effect from the resumption date set out in the amendment. This date may be before the date on which the amendment enters into force.

Costs incurred during suspension of the action implementation are not eligible (see Article 6).

## 49.2 Suspension of the action implementation, by the Commission

### 49.2.1 Conditions

The Commission may suspend implementation of the action or any part of it, if:

- (a) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed or is suspected of having committed:
  - (i) substantial errors, irregularities or fraud or
  - (ii) serious breach of obligations under the Agreement or during the award procedure (including improper implementation of the action, submission of false information, failure to provide required information, breach of ethical principles);
- (b) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed — in other EU or Euratom grants awarded to it under similar conditions — systemic or recurrent errors, irregularities, fraud or serious breach of obligations that have a material impact on this grant (**extension of findings from other grants to this grant**; see Article 22.5.2), or
- (c) the action is suspected of having lost its scientific or technological relevance.

### 49.2.2 Procedure

Before suspending implementation of the action, the Commission will formally notify the coordinator or beneficiary concerned:

- informing it of its intention to suspend the implementation and the reasons why and
- inviting it to submit observations within 30 days of receiving notification.

If the Commission does not receive observations or decides to pursue the procedure despite the observations it has received, it will formally notify **confirmation** of the suspension. Otherwise, it will formally notify that the procedure is not continued.

The suspension will **take effect** five days after confirmation notification is received (or on a later date specified in the notification).

It will be **lifted** if the conditions for resuming implementation of the action are met.

The coordinator or beneficiary concerned will be formally notified of the lifting and the Agreement will be **amended** to set the date on which the action will be resumed, extend the duration of the action and make other changes necessary to adapt the action to the new situation (see Article 55) — unless the Agreement has already been terminated (see Article 50).

The suspension will be lifted with effect from the resumption date set out in the amendment. This date may be before the date on which the amendment enters into force.

Costs incurred during suspension are not eligible (see Article 6).

The beneficiaries may not claim damages due to suspension by the Commission (see Article 46).

Suspension of the action implementation does not affect the Commission's right to terminate the Agreement or participation of a beneficiary (see Article 50), reduce the grant or recover amounts unduly paid (see Articles 43 and 44).

## **ARTICLE 50 — TERMINATION OF THE AGREEMENT OR OF THE PARTICIPATION OF ONE OR MORE BENEFICIARIES**

### **50.1 Termination of the Agreement, by the beneficiaries**

#### **50.1.1 Conditions and procedure**

The beneficiaries may terminate the Agreement.

The coordinator must formally notify termination to the Commission (see Article 52), stating:

- the reasons why and
- the date the termination will take effect. This date must be after the notification.

If no reasons are given or if the Commission considers the reasons do not justify termination, the Agreement will be considered to have been '**terminated improperly**'.

The termination will **take effect** on the day specified in the notification.

#### **50.1.2 Effects**

The coordinator must — within 60 days from when termination takes effect — submit:

- (i) a periodic report (for the open reporting period until termination; see Article 20.3) and
- (ii) the final report (see Article 20.4).

If the Commission does not receive the reports within the deadline (see above), only costs which are included in an approved periodic report will be taken into account.

The Commission will **calculate** the final grant amount (see Article 5.3) and the balance (see Article 21.4) on the basis of the reports submitted. Only costs incurred until termination are eligible (see Article 6). Costs relating to contracts due for execution only after termination are not eligible.

Improper termination may lead to a reduction of the grant (see Article 43).

After termination, the beneficiaries' obligations (in particular Articles 20, 22, 23, Section 3 of Chapter 4, 36, 37, 38, 40, 42, 43 and 44) continue to apply.

### **50.2 Termination of the participation of one or more beneficiaries, by the beneficiaries**

#### **50.2.1 Conditions and procedure**

The participation of one or more beneficiaries may be terminated by the coordinator, on request of the beneficiary concerned or on behalf of the other beneficiaries.

The coordinator must formally notify termination to the Commission (see Article 52) and inform the beneficiary concerned.

If the coordinator's participation is terminated without its agreement, the formal notification must be done by another beneficiary (acting on behalf of the other beneficiaries).

The notification must include:

- the reasons why;
- the opinion of the beneficiary concerned (or proof that this opinion has been requested in writing);
- the date the termination takes effect. This date must be after the notification, and
- a request for amendment (see Article 55), with a proposal for reallocation of the tasks and the estimated budget of the beneficiary concerned (see Annexes 1 and 2) and, if necessary, the addition of one or more new beneficiaries (see Article 56). If termination takes effect after the period set out in Article 3, no request for amendment must be included unless the beneficiary concerned is the coordinator. In this case, the request for amendment must propose a new coordinator.

If this information is not given or if the Commission considers that the reasons do not justify termination, the participation will be considered to have been **terminated improperly**.

The termination will **take effect** on the day specified in the notification.

### 50.2.2 Effects

The coordinator must — within 30 days from when termination takes effect — submit:

- (i) a report on the distribution of payments to the beneficiary concerned and
- (ii) if termination takes effect during the period set out in Article 3, a '**termination report**' from the beneficiary concerned, for the open reporting period until termination, containing an overview of the progress of the work, an overview of the use of resources, the individual financial statement and, if applicable, the certificate on the financial statement (see Articles 20.3 and 20.4).

The information in the termination report must also be included in the periodic report for the next reporting period (see Article 20.3).

If the request for amendment is rejected by the Commission (because it calls into question the decision awarding the grant or breaches the principle of equal treatment of applicants), the Agreement may be terminated according to Article 50.3.1(c).

If the request for amendment is accepted by the Commission, the Agreement is **amended** to introduce the necessary changes (see Article 55).

The Commission will — on the basis of the periodic reports, the termination report and the report

on the distribution of payments — **calculate** the amount which is due to the beneficiary and if the (pre-financing and interim) payments received by the beneficiary exceed this amount.

The **amount which is due** is calculated in the following steps:

Step 1 — Application of the reimbursement rate to the eligible costs

The grant amount for the beneficiary is calculated by applying the reimbursement rate(s) to the total eligible costs declared by the beneficiary in the termination report and approved by the Commission.

Only costs incurred by the beneficiary concerned until termination takes effect are eligible (see Article 6). Costs relating to contracts due for execution only after termination are not eligible.

Step 2 — Reduction due to substantial errors, irregularities or fraud or serious breach of obligations

In case of a reduction (see Article 43), the Commission will calculate the reduced grant amount for the beneficiary by deducting the amount of the reduction (calculated in proportion to the seriousness of the errors, irregularities or fraud or breach of obligations, in accordance with Article 43.2) from the grant amount for the beneficiary.

If the payments received **exceed the amounts due**:

- if termination takes effect during the period set out in Article 3 and the request for amendment is accepted, the beneficiary concerned must repay to the coordinator the amount unduly received. The Commission will formally notify the amount unduly received and request the beneficiary concerned to repay it to the coordinator within 30 days of receiving notification. If it does not repay the coordinator, the Commission will draw upon the Guarantee Fund to pay the coordinator and then notify a **debit note** on behalf of the Guarantee Fund to the beneficiary concerned (see Article 44);
- in all other cases, in particular if termination takes effect after the period set out in Article 3, the Commission will formally notify a **debit note** to the beneficiary concerned. If payment is not made by the date in the debit note, the Guarantee Fund will pay to the Commission the amount due and the Commission will notify a debit note on behalf of the Guarantee Fund to the beneficiary concerned (see Article 44);
- if the beneficiary concerned is the former coordinator, it must repay the new coordinator according to the procedure above, unless:
  - termination takes effect after an interim payment and
  - the former coordinator has not distributed amounts received as pre-financing or interim payments (see Article 21.7).

In this case, the Commission will formally notify a **debit note** to the former coordinator. If payment is not made by the date in the debit note, the Guarantee Fund will pay to the Commission the amount due. The Commission will then pay the new coordinator and notify a debit note on behalf of the Guarantee Fund to the former coordinator (see Article 44).

If the payments received **do not exceed the amounts due**: amounts owed to the beneficiary concerned will be included in the next interim or final payment.

If the Commission does not receive the termination report within the deadline (see above), only costs included in an approved periodic report will be taken into account.

If the Commission does not receive the report on the distribution of payments within the deadline (see above), it will consider that:

- the coordinator did not distribute any payment to the beneficiary concerned and that
- the beneficiary concerned must not repay any amount to the coordinator.

Improper termination may lead to a reduction of the grant (see Article 43) or termination of the Agreement (see Article 50).

After termination, the concerned beneficiary's obligations (in particular Articles 20, 22, 23, Section 3 of Chapter 4, 36, 37, 38, 40, 42, 43 and 44) continue to apply.

### **50.3 Termination of the Agreement or the participation of one or more beneficiaries, by the Commission**

#### **50.3.1 Conditions**

The Commission may terminate the Agreement or the participation of one or more beneficiaries, if:

- (a) one or more beneficiaries do not accede to the Agreement (see Article 56);
- (b) a change to their legal, financial, technical, organisational or ownership situation is likely to substantially affect or delay the implementation of the action or calls into question the decision to award the grant;
- (c) following termination of participation for one or more beneficiaries (see above), the necessary changes to the Agreement would call into question the decision awarding the grant or breach the principle of equal treatment of applicants (see Article 55);
- (d) implementation of the action is prevented by force majeure (see Article 51) or suspended by the coordinator (see Article 49.1) and either:
  - (i) resumption is impossible, or
  - (ii) the necessary changes to the Agreement would call into question the decision awarding the grant or breach the principle of equal treatment of applicants;
- (e) a beneficiary is declared bankrupt, being wound up, having its affairs administered by the courts, has entered into an arrangement with creditors, has suspended business activities, or is subject to any other similar proceedings or procedures under national law;
- (f) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has been found guilty of professional misconduct, proven by any means;
- (g) a beneficiary does not comply with the applicable national law on taxes and social security;

- (h) the action has lost scientific or technological relevance;
- (i) not applicable;
- (j) not applicable;
- (k) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed fraud, corruption, or is involved in a criminal organisation, money laundering or any other illegal activity;
- (l) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed:
  - (i) substantial errors, irregularities or fraud or
  - (ii) serious breach of obligations under the Agreement or during the award procedure (including improper implementation of the action, submission of false information, failure to provide required information, breach of ethical principles);
- (m) a beneficiary (or a natural person who has the power to represent or take decisions on its behalf) has committed — in other EU or Euratom grants awarded to it under similar conditions — systemic or recurrent errors, irregularities, fraud or serious breach of obligations that have a material impact on this grant (**extension of findings from other grants to this grant**; see Article 22.5.2);
- (n) despite a specific request by the Commission, a beneficiary does not request — through the coordinator — an amendment to the Agreement to end the participation of one of its linked third parties or international partners that is in one of the situations under points (e), (f), (g), (k), (l) or (m) and to reallocate its tasks.

### 50.3.2 Procedure

Before terminating the Agreement or participation of one or more beneficiaries, the Commission will formally notify the coordinator or beneficiary concerned:

- informing it of its intention to terminate and the reasons why and
- inviting it, within 30 days of receiving notification, to submit observations and — in case of Point (l.ii) above — to inform the Commission of the measures to ensure compliance with the obligations under the Agreement.

If the Commission does not receive observations or decides to pursue the procedure despite the observations it has received, it will formally notify to the coordinator or beneficiary concerned **confirmation** of the termination and the date it will take effect. Otherwise, it will formally notify that the procedure is not continued.

The termination will **take effect**:

- for terminations under Points (b), (c), (e), (g), (h), (j), (l.ii) and (n) above: on the day specified in the notification of the confirmation (see above);
- for terminations under Points (a), (d), (f), (i), (k), (l.i) and (m) above: on the day after the notification of the confirmation is received.



### 50.3.3 Effects

#### (a) for **termination of the Agreement**:

The coordinator must — within 60 days from when termination takes effect — submit:

- (i) a periodic report (for the last open reporting period until termination; see Article 20.3) and
- (ii) a final report (see Article 20.4).

If the Agreement is terminated for breach of the obligation to submit reports (see Articles 20.8 and 50.3.1(l)), the coordinator may not submit any reports after termination.

If the Commission does not receive the reports within the deadline (see above), only costs which are included in an approved periodic report will be taken into account.

The Commission will **calculate** the final grant amount (see Article 5.3) and the balance (see Article 21.4) on the basis of the reports submitted. Only costs incurred until termination takes effect are eligible (see Article 6). Costs relating to contracts due for execution only after termination are not eligible.

This does not affect the Commission's right to reduce the grant (see Article 43) or to impose administrative sanctions (Article 45).

The beneficiaries may not claim damages due to termination by the Commission (see Article 46).

After termination, the beneficiaries' obligations (in particular Articles 20, 22, 23, Section 3 of Chapter 4, 36, 37, 38, 40, 42, 43 and 44) continue to apply.

#### (b) for **termination of the participation of one or more beneficiaries**:

The coordinator must — within 60 days from when termination takes effect — submit:

- (i) a report on the distribution of payments to the beneficiary concerned;
- (ii) a request for amendment (see Article 55), with a proposal for reallocation of the tasks and estimated budget of the beneficiary concerned (see Annexes 1 and 2) and, if necessary, the addition of one or more new beneficiaries (see Article 56). If termination is notified after the period set out in Article 3, no request for amendment must be submitted unless the beneficiary concerned is the coordinator. In this case the request for amendment must propose a new coordinator, and
- (iii) if termination takes effect during the period set out in Article 3, a **termination report** from the beneficiary concerned, for the open reporting period until termination, containing an overview of the progress of the work, an overview of the use of resources, the individual financial statement and, if applicable, the certificate on the financial statement (see Article 20).

The information in the termination report must also be included in the periodic report for the next reporting period (see Article 20.3).



If the request for amendment is rejected by the Commission (because it calls into question the decision awarding the grant or breaches the principle of equal treatment of applicants), the Agreement may be terminated according to Article 50.3.1(c).

If the request for amendment is accepted by the Commission, the Agreement is **amended** to introduce the necessary changes (see Article 55).

The Commission will — on the basis of the periodic reports, the termination report and the report on the distribution of payments — **calculate** the amount which is due to the beneficiary and if the (pre-financing and interim) payments received by the beneficiary exceed this amount.

The **amount which is due** is calculated in the following steps:

Step 1 — Application of the reimbursement rate to the eligible costs

The grant amount for the beneficiary is calculated by applying the reimbursement rate(s) to the total eligible costs declared by the beneficiary in the termination report and approved by the Commission.

Only costs incurred by the beneficiary concerned until termination takes effect are eligible (see Article 6). Costs relating to contracts due for execution only after termination are not eligible.

Step 2 — Reduction due to substantial errors, irregularities or fraud or serious breach of obligations

In case of a reduction (see Article 43), the Commission will calculate the reduced grant amount for the beneficiary by deducting the amount of the reduction (calculated in proportion to the seriousness of the errors, irregularities or fraud or breach of obligations, in accordance with Article 43.2) from the grant amount for the beneficiary.

If the payments received **exceed the amounts due**:

- if termination takes effect during the period set out in Article 3 and the request for amendment is accepted, the beneficiary concerned must repay to the coordinator the amount unduly received. The Commission will formally notify the amount unduly received and request the beneficiary concerned to repay it to the coordinator within 30 days of receiving notification. If it does not repay the coordinator, the Commission will draw upon the Guarantee Fund to pay the coordinator and then notify a **debit note** on behalf of the Guarantee Fund to the beneficiary concerned (see Article 44);
- in all other cases, in particular if termination takes effect after the period set out in Article 3, the Commission will formally notify a **debit note** to the beneficiary concerned. If payment is not made by the date in the debit note, the Guarantee Fund will pay to the Commission the amount due and the Commission will notify a debit note on behalf of the Guarantee Fund to the beneficiary concerned (see Article 44);
- if the beneficiary concerned is the former coordinator, it must repay the new coordinator according to the procedure above, unless:

- termination takes effect after an interim payment and
- the former coordinator has not distributed amounts received as pre-financing or interim payments (see Article 21.7).

In this case, the Commission will formally notify a **debit note** to the former coordinator. If payment is not made by the date in the debit note, the Guarantee Fund will pay to the Commission the amount due. The Commission will then pay the new coordinator and notify a debit note on behalf of the Guarantee Fund to the former coordinator (see Article 44).

If the payments received **do not exceed the amounts due**: amounts owed to the beneficiary concerned will be included in the next interim or final payment.

If the Commission does not receive the termination report within the deadline (see above), only costs included in an approved periodic report will be taken into account.

If the Commission does not receive the report on the distribution of payments within the deadline (see above), it will consider that:

- the coordinator did not distribute any payment to the beneficiary concerned and that
- the beneficiary concerned must not repay any amount to the coordinator.

After termination, the concerned beneficiary's obligations (in particular Articles 20, 22, 23, Section 3 of Chapter 4, 36, 37, 38, 40, 42, 43 and 44) continue to apply.

## **SECTION 4 FORCE MAJEURE**

### **ARTICLE 51 — FORCE MAJEURE**

'Force majeure' means any situation or event that:

- prevents either party from fulfilling their obligations under the Agreement,
- was unforeseeable, exceptional situation and beyond the parties' control,
- was not due to error or negligence on their part (or on the part of third parties involved in the action), and
- proves to be inevitable in spite of exercising all due diligence.

The following cannot be invoked as force majeure:

- any default of a service, defect in equipment or material or delays in making them available, unless they stem directly from a relevant case of force majeure,
- labour disputes or strikes, or
- financial difficulties.

Any situation constituting force majeure must be formally notified to the other party without delay, stating the nature, likely duration and foreseeable effects.

The parties must immediately take all the necessary steps to limit any damage due to force majeure and do their best to resume implementation of the action as soon as possible.

The party prevented by force majeure from fulfilling its obligations under the Agreement cannot be considered in breach of them.

## **CHAPTER 7 FINAL PROVISIONS**

### **ARTICLE 52 — COMMUNICATION BETWEEN THE PARTIES**

#### **52.1 Form and means of communication**

Communication under the Agreement (information, requests, submissions, ‘formal notifications’, etc.) must:

- be made in writing and
- bear the number of the Agreement.

All communication must be made through the Participant Portal **electronic** exchange system and using the forms and templates provided there.

If — after the payment of the balance — the Commission finds that a formal notification was not accessed, a second formal notification will be made by registered post with proof of delivery (‘formal notification on **paper**’). Deadlines will be calculated from the moment of the second notification.

Communications in the electronic exchange system must be made by persons authorised according to the Participant Portal Terms & Conditions. For naming the authorised persons, each beneficiary must have designated — before the signature of this Agreement — a ‘legal entity appointed representative (LEAR)’. The role and tasks of the LEAR are stipulated in his/her appointment letter (see Participant Portal Terms & Conditions).

If the electronic exchange system is temporarily unavailable, instructions will be given on the Commission website.

#### **52.2 Date of communication**

**Communications** are considered to have been made when they are sent by the sending party (i.e. on the date and time they are sent through the electronic exchange system).

**Formal notifications** through the **electronic** exchange system are considered to have been made when they are received by the receiving party (i.e. on the date and time of acceptance by the receiving party, as indicated by the time stamp). A formal notification that has not been accepted within 10 days after sending is considered to have been accepted.

Formal notifications **on paper** sent by **registered post** with proof of delivery (only after the payment of the balance) are considered to have been made on either:

- the delivery date registered by the postal service or
- the deadline for collection at the post office.

If the electronic exchange system is temporarily unavailable, the sending party cannot be considered in breach of its obligation to send a communication within a specified deadline.

### 52.3 Addresses for communication

The **electronic** exchange system must be accessed via the following URL:

<https://ec.europa.eu/research/participants/portal/desktop/en/projects/>

The Commission will formally notify the coordinator and beneficiaries in advance any changes to this URL.

**Formal notifications on paper** (only after the payment of the balance) addressed **to the Commission** must be sent to the official mailing address indicated on the Commission's website.

Formal notifications on paper (only after the payment of the balance) addressed **to the beneficiaries** must be sent to their legal address as specified in the Participant Portal Beneficiary Register.

## ARTICLE 53 — INTERPRETATION OF THE AGREEMENT

### 53.1 Precedence of the Terms and Conditions over the Annexes

The provisions in the Terms and Conditions of the Agreement take precedence over its Annexes.

Annex 2 takes precedence over Annex 1.

### 53.2 Privileges and immunities

Not applicable

## ARTICLE 54 — CALCULATION OF PERIODS, DATES AND DEADLINES

In accordance with Regulation No 1182/71<sup>30</sup>, periods expressed in days, months or years are calculated from the moment the triggering event occurs.

The day during which that event occurs is not considered as falling within the period.

## ARTICLE 55 — AMENDMENTS TO THE AGREEMENT

### 55.1 Conditions

The Agreement may be amended, unless the amendment entails changes to the Agreement which would call into question the decision awarding the grant or breach the principle of equal treatment of applicants.

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<sup>30</sup> Regulation (EEC, Euratom) No 1182/71 of the Council of 3 June 1971 determining the rules applicable to periods, dates and time-limits (OJ L 124, 8.6.1971, p. 1).

Amendments may be requested by any of the parties.

## 55.2 Procedure

The party requesting an amendment must submit a request for amendment signed in the electronic exchange system (see Article 52).

The coordinator submits and receives requests for amendment on behalf of the beneficiaries (see Annex 3).

If a change of coordinator is requested without its agreement, the submission must be done by another beneficiary (acting on behalf of the other beneficiaries).

The request for amendment must include:

- the reasons why;
- the appropriate supporting documents, and
- for a change of coordinator without its agreement: the opinion of the coordinator (or proof that this opinion has been requested in writing).

The Commission may request additional information.

If the party receiving the request agrees, it must sign the amendment in the electronic exchange system within 45 days of receiving notification (or any additional information the Commission has requested). If it does not agree, it must formally notify its disagreement within the same deadline. The deadline may be extended, if necessary for the assessment of the request. If no notification is received within the deadline, the request is considered to have been rejected

An amendment **enters into force** on the day of the signature of the receiving party.

An amendment **takes effect** on the date agreed by the parties or, in the absence of such an agreement, on the date on which the amendment enters into force.

## ARTICLE 56 — ACCESSION TO THE AGREEMENT

### 56.1 Accession of the beneficiaries mentioned in the Preamble

The other beneficiaries must accede to the Agreement by signing the Accession Form (see Annex 3) in the electronic exchange system (see Article 52) within 30 days after its entry into force (see Article 58).

They will assume the rights and obligations under the Agreement with effect from the date of its entry into force (see Article 58).

If a beneficiary does not accede to the Agreement within the above deadline, the coordinator must — within 30 days — request an amendment to make any changes necessary to ensure proper implementation of the action. This does not affect the Commission's right to terminate the Agreement (see Article 50).

### 56.2 Addition of new beneficiaries

In justified cases, the beneficiaries may request the addition of a new beneficiary.

For this purpose, the coordinator must submit a request for amendment in accordance with Article 55. It must include an Accession Form (see Annex 3) signed by the new beneficiary in the electronic exchange system (see Article 52).

New beneficiaries must assume the rights and obligations under the Agreement with effect from the date of their accession specified in the Accession Form (see Annex 3).

## **ARTICLE 57 — APPLICABLE LAW AND SETTLEMENT OF DISPUTES**

### **57.1 Applicable law**

The Agreement is governed by the applicable EU law, supplemented if necessary by the law of Belgium.

### **57.2 Dispute settlement**

If a dispute concerning the interpretation, application or validity of the Agreement cannot be settled amicably, the General Court — or, on appeal, the Court of Justice of the European Union — has sole jurisdiction. Such actions must be brought under Article 272 of the Treaty on the Functioning of the EU (TFEU).

As an exception, if such a dispute is between the Commission and INSTITUT ZA MOLEKULARNU GENETIKU I GENETICKO INZENJERSTVO, the competent Belgian courts have sole jurisdiction.

If a dispute concerns administrative sanctions, offsetting or an enforceable decision under Article 299 TFEU (see Articles 44, 45 and 46), the beneficiaries must bring action before the General Court — or, on appeal, the Court of Justice of the European Union — under Article 263 TFEU.

## **ARTICLE 58 — ENTRY INTO FORCE OF THE AGREEMENT**

The Agreement will enter into force on the day of signature by the Commission or the coordinator, depending on which is later.

### **SIGNATURES**

For the coordinator

For the Commission



**EUROPEAN COMMISSION**  
Directorate-General for Research and Innovation  
Sustainable Industry Systems



## **ANNEX 1 (part A)**

**Research and Innovation action**

**NUMBER — 870292 — BioICEP**



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# 1.1. The project summary

Project Number <sup>1</sup>	870292	Project Acronym <sup>2</sup>	BioICEP
One form per project			
General information			
Project title <sup>3</sup>	Bio Innovation of a Circular Economy for Plastics		
Starting date <sup>4</sup>	01/01/2020		
Duration in months <sup>5</sup>	48		
Call (part) identifier <sup>6</sup>	H2020-NMBP-BIO-CN-2019		
Topic	CE-BIOTEC-05-2019 Microorganism communities for plastics bio-degradation (RIA)		
Fixed EC Keywords	Industrial bioengineering, Synthetic biology, chemical biology and new bio-engineering concepts, Environmental and marine biology, Cell biology, Microbiology, Biological engineering		
Free keywords	Biocatalysis, mechano-biochemical, microbial consortia, depolymerisation, bioproducts, bioplastics, bioprocessing, circular economy for plastics, low greenhouse gas emissions, sustainable		
Abstract <sup>7</sup>			
<p>The Bio Innovation of a Circular Economy for Plastics (BioICEP) is a pan European-Chinese collaboration formed to reduce the burden of plastic waste in the environment. Different mixed plastic pollution environments are represented, with specific partners selected which have the expertise and facilities to carry out the necessary technical innovations. A number of innovative booster technologies are at the core of this solution accentuating, expediting, and augmenting mixed plastics degradation to levels far in excess of those current achievable. Our approach is The Bio Innovation of a Circular Economy for Plastics (BioICEP) consortium is a pan European-Chinese collaborative formed to reduce the burden of plastic waste in the environment. The countries have been selected to represent different mixed plastic pollution environments, with specific partners selected which have the expertise and facilities to carry out the necessary technical innovations. Three innovative booster technologies are at the core of this solution accentuating, expediting, and augmenting plastics degradation to levels far in excess of those current achievable. Our approach is a triple-action depolymerisation system where plastic waste will be broken down in three consecutive processes: 1) mechano-biochemical disintegration processes, including a new proprietary sonic-green-chemical technology to reduce the polymer molecular weight of the base polymer to make it amenable to biodegradation; 2) biocatalytic digestion, with enzymes enhanced through a range of innovative techniques including accelerated screening through novel fluorescent sensor and directed evolution; and 3) microbial consortia developed from best in class single microbial strains, which combined leads to highly efficient degradation of mixed plastic waste streams. The outputs from this degradation process will be used as building blocks for new polymers or other bioproducts to enable a new plastic waste-based circular economy.</p>			

## 1.2. List of Beneficiaries

 Associated with document Ref. Ares(2019)6080743 - 01/10/2019

Project Number <sup>1</sup>	870292	Project Acronym <sup>2</sup>	BioICEP
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### List of Beneficiaries

No	Name	Short name	Country	Project entry month <sup>8</sup>	Project exit month
1	ATHLONE INSTITUTE OF TECHNOLOGY	AIT	Ireland	1	48
2	ACTECO PRODUCTOS Y SERVICIOS SL	ACTECO	Spain	1	48
3	AIMPLAS - ASOCIACION DE INVESTIGACION DE MATERIALES PLASTICOS Y CONEXAS	AIMPLAS	Spain	1	48
4	AVECOM	AVECOM	Belgium	1	48
5	TECHNISCHE UNIVERSITAT CLAUSTHAL	TUC	Germany	1	48
6	INSTITUT ZA MOLEKULARNU GENETIKU I GENETICKO INZENJERSTVO	IMGGE	Serbia	1	48
7	INSTITUTO DE BIOLOGIA EXPERIMENTAL E TECNOLÓGICA	IBET	Portugal	1	48
8	LIMERICK INSTITUTE OF TECHNOLOGY	LIT	Ireland	1	48
9	LOGOPLASTE INNOVATION LAB LDA	LOGOPLASTE	Portugal	1	48
10	MICROLIFE SOLUTIONS BV	MicroLife	Netherlands	1	48
11	NATIONAL TECHNICAL UNIVERSITY OF ATHENS - NTUA	NTUA	Greece	1	48
12	THE PROVOST, FELLOWS, FOUNDATION SCHOLARS & THE OTHER MEMBERS OF BOARD OF THE COLLEGE OF THE HOLY & UNDIVIDED TRINITY OF QUEEN ELIZABETH NEAR DUBLIN	TCD	Ireland	1	48

## 1.3. Workplan Tables - Detailed Implementation

### 1.3.1. WT1 List of work packages

WP Number <sup>9</sup>	WP Title	Lead beneficiary <sup>10</sup>	Person-months <sup>11</sup>	Start month <sup>12</sup>	End month <sup>13</sup>
WP1	Ethics requirements	1 - AIT	N/A	1	48
WP2	Project Management and Coordination	1 - AIT	61.50	1	48
WP3	Pre-Treatment Processes for Plastic Bio-Degradation	12 - TCD	127.00	2	48
WP4	Development of Enzymatic and Biocatalytic Solutions for Single and Mixed Plastic Degradation	6 - IMGGE	123.00	2	46
WP5	Establishment of a catalogue of high performance microbial strains for plastic degradation and bioplastic production	8 - LIT	96.00	2	38
WP6	Establishment of high performance microbial consortia for plastic degradation and bioplastic production	10 - MicroLife	77.00	4	46
WP7	Bioprocess Development for Production of Value-added Biopolymer Products	7 - IBET	126.00	8	48
WP8	Establishment BioICEP Pilot plant for mixed plastics degradation and bioproduct production	4 - AVECOM	62.00	12	48
WP9	Dissemination, Exploitation, and Communication	3 - AIMPLAS	55.00	1	48
WP10	Ethics requirements	1 - AIT	1.00	1	48
<b>Total</b>			728.50		

### 1.3.2. WT2 list of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	WP number <sup>9</sup>	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D1.1	NEC - Requirement No. 1	WP1	1 - AIT	Ethics	Confidential, only for members of the consortium (including the Commission Services)	6
D1.2	EPQ - Requirement No. 2	WP1	1 - AIT	Ethics	Confidential, only for members of the consortium (including the Commission Services)	1
D2.1	Internet based communication platform and repository	WP2	1 - AIT	Websites, patents filling, etc.	Confidential, only for members of the consortium (including the Commission Services)	2
D2.2	Technical management	WP2	1 - AIT	data sets, microdata, etc	Confidential, only for members of the consortium (including the Commission Services)	3
D2.3	Report with all Executive Board meetings	WP2	1 - AIT	Report	Confidential, only for members of the consortium (including the Commission Services)	48
D3.1	Plastic waste partner supply report	WP3	12 - TCD	Report	Confidential, only for members of the consortium (including the Commission Services)	6
D3.2	New plastic waste pre-treatment process report	WP3	12 - TCD	Report	Confidential, only for members of the consortium (including the Commission Services)	24
D3.3	Report on degraded plastic waste based compatabilisers	WP3	12 - TCD	Report	Confidential, only for members of the consortium (including the Commission Services)	48

<b>Deliverable Number<sup>14</sup></b>	<b>Deliverable Title</b>	<b>WP number<sup>9</sup></b>	<b>Lead beneficiary</b>	<b>Type<sup>15</sup></b>	<b>Dissemination level<sup>16</sup></b>	<b>Due Date (in months)<sup>17</sup></b>
D4.1	Report on the synthesis of model compounds	WP4	6 - IMGGE	Report	Confidential, only for members of the consortium (including the Commission Services)	12
D4.2	Demonstration of new accelerated Screening	WP4	6 - IMGGE	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	24
D4.3	Report on mechanism of enzymatic and/or microbial attack	WP4	6 - IMGGE	Report	Confidential, only for members of the consortium (including the Commission Services)	24
D4.4	Report on discovery of at least four novel enzymatic activities	WP4	6 - IMGGE	Report	Confidential, only for members of the consortium (including the Commission Services)	30
D4.5	Report on Enzymatic Immobilization	WP4	6 - IMGGE	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D4.6	Biocatalyst improvement by directed evolution	WP4	11 - NTUA	Report	Confidential, only for members of the consortium (including the Commission Services)	40
D4.7	Construction of the microbial cell factory	WP4	6 - IMGGE	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	46
D5.1	Identification of efficient microbial plastic degraders	WP5	8 - LIT	Report	Confidential, only for members of the consortium (including the Commission Services)	20

<b>Deliverable Number<sup>14</sup></b>	<b>Deliverable Title</b>	<b>WP number<sup>9</sup></b>	<b>Lead beneficiary</b>	<b>Type<sup>15</sup></b>	<b>Dissemination level<sup>16</sup></b>	<b>Due Date (in months)<sup>17</sup></b>
D5.2	Generation of novel boosted degradation capacity strains	WP5	8 - LIT	data sets, microdata, etc	Confidential, only for members of the consortium (including the Commission Services)	24
D5.3	Liquid cultivation conditions for pre-identified degraders	WP5	8 - LIT	Report	Confidential, only for members of the consortium (including the Commission Services)	28
D5.4	Characterisation of depolymerase enzyme activities	WP5	6 - IMGGE	Report	Confidential, only for members of the consortium (including the Commission Services)	33
D5.5	Quantitative/ qualitative analysis of plastic breakdown characterisation	WP5	8 - LIT	Report	Confidential, only for members of the consortium (including the Commission Services)	34
D5.6	PHB, rhamnolipids and nanocellulose producers	WP5	8 - LIT	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D5.7	Recommended strains for WP3, WP5 and WP6	WP5	8 - LIT	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D6.1	Report on enrichment of existing microbial communities	WP6	10 - MicroLife	Report	Confidential, only for members of the consortium (including the Commission Services)	18
D6.2	Report on synthetic community vs individual performance	WP6	10 - MicroLife	Report	Confidential, only for members of the consortium (including the Commission Services)	24
D6.3	Identification of degrading enzymes	WP6	10 - MicroLife	Report	Confidential, only for members	30

<b>Deliverable Number<sup>14</sup></b>	<b>Deliverable Title</b>	<b>WP number<sup>9</sup></b>	<b>Lead beneficiary</b>	<b>Type<sup>15</sup></b>	<b>Dissemination level<sup>16</sup></b>	<b>Due Date (in months)<sup>17</sup></b>
	within best performing communities				of the consortium (including the Commission Services)	
D6.4	Optimized synthetic and natural microbial communities	WP6	10 - MicroLife	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D6.5	Information on plastic breakdown products for WP6	WP6	1 - AIT	Report	Confidential, only for members of the consortium (including the Commission Services)	42
D7.1	Report on the best strains and process operation conditions	WP7	7 - IBET	Report	Confidential, only for members of the consortium (including the Commission Services)	20
D7.2	Report on the metabolic model and monitoring	WP7	7 - IBET	Report	Confidential, only for members of the consortium (including the Commission Services)	38
D7.3	Report on optimised PHBs, nanocellulose and rhamnolipids	WP7	1 - AIT	Report	Confidential, only for members of the consortium (including the Commission Services)	44
D7.4	Protocols on the optimized conditions for downstream process	WP7	7 - IBET	Report	Confidential, only for members of the consortium (including the Commission Services)	48
D8.1	Operation of modular integrated pilot scale plant	WP8	4 - AVECOM	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D8.2	Pilot production of high performance PHB and nanocellulose	WP8	4 - AVECOM	Report	Confidential, only for members of the consortium (including the	40



<b>Deliverable Number<sup>14</sup></b>	<b>Deliverable Title</b>	<b>WP number<sup>9</sup></b>	<b>Lead beneficiary</b>	<b>Type<sup>15</sup></b>	<b>Dissemination level<sup>16</sup></b>	<b>Due Date (in months)<sup>17</sup></b>
					Commission Services)	
D8.3	Report on Life Cycle Analysis study	WP8	12 - TCD	Report	Confidential, only for members of the consortium (including the Commission Services)	44
D9.1	Project website	WP9	3 - AIMPLAS	Websites, patents filling, etc.	Public	2
D9.2	Communication Plan (CP)	WP9	3 - AIMPLAS	Websites, patents filling, etc.	Confidential, only for members of the consortium (including the Commission Services)	4
D9.3	Data Management Plan (DMP)	WP9	3 - AIMPLAS	Report	Confidential, only for members of the consortium (including the Commission Services)	6
D9.4	Preliminary Plan for Dissemination and Exploitation of Results progress (PDER)	WP9	3 - AIMPLAS	Report	Confidential, only for members of the consortium (including the Commission Services)	12
D9.5	PDER	WP9	3 - AIMPLAS	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D9.6	Open Research Data Pilot	WP9	1 - AIT	ORDP: Open Research Data Pilot	Public	6
D9.7	Technology Watch Service report	WP9	1 - AIT	Report	Confidential, only for members of the consortium (including the Commission Services)	48
D9.8	Business Model presenting the go to market potential	WP9	1 - AIT	Report	Confidential, only for members of the consortium (including the	48

<b>Deliverable Number<sup>14</sup></b>	<b>Deliverable Title</b>	<b>WP number<sup>9</sup></b>	<b>Lead beneficiary</b>	<b>Type<sup>15</sup></b>	<b>Dissemination level<sup>16</sup></b>	<b>Due Date (in months)<sup>17</sup></b>
					Commission Services)	
D10.1	NEC Requirement 1	WP10	1 - AIT	Other	Confidential, only for members of the consortium (including the Commission Services)	3
D10.2	EPQ- Requirement No. 2	WP10	1 - AIT	Report	Confidential, only for members of the consortium (including the Commission Services)	48

### 1.3.3. WT3 Work package descriptions

<b>Work package number</b> <sup>9</sup>	WP1	<b>Lead beneficiary</b> <sup>10</sup>	1 - AIT
<b>Work package title</b>	Ethics requirements		
<b>Start month</b>	1	<b>End month</b>	48

#### Objectives

The objective is to ensure compliance with the 'ethics requirements' set out in this work package.

#### Description of work and role of partners

**WP1 - Ethics requirements** [Months: 1-48]

**AIT**

This work package sets out the 'ethics requirements' that the project must comply with.

#### List of deliverables

<b>Deliverable Number</b> <sup>14</sup>	<b>Deliverable Title</b>	<b>Lead beneficiary</b>	<b>Type</b> <sup>15</sup>	<b>Dissemination level</b> <sup>16</sup>	<b>Due Date (in months)</b> <sup>17</sup>
D1.1	NEC - Requirement No. 1	1 - AIT	Ethics	Confidential, only for members of the consortium (including the Commission Services)	6
D1.2	EPQ - Requirement No. 2	1 - AIT	Ethics	Confidential, only for members of the consortium (including the Commission Services)	1

#### Description of deliverables

The 'ethics requirements' that the project must comply with are included as deliverables in this work package.

D1.1 : NEC - Requirement No. 1 [6]

1. In case activities undertaken in non-EU countries raise ethics issues, the applicants must ensure that the research conducted outside the EU is legal in at least one EU Member State. This must be submitted as a deliverable. 2. Details on the materials which will be imported to/exported from the EU must be kept on file and submitted as a deliverable. 3. Copies of import/export authorisations, as required by national/EU legislation must be kept on file.

D1.2 : EPQ - Requirement No. 2 [1]

7.1. Further information about the possible harm to the environment caused by the research and the measures that will be taken to mitigate the risks must be kept on file and submitted as a deliverable.

#### Schedule of relevant Milestones

<b>Milestone number</b> <sup>18</sup>	<b>Milestone title</b>	<b>Lead beneficiary</b>	<b>Due Date (in months)</b>	<b>Means of verification</b>
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<b>Work package number</b> <sup>9</sup>	WP2	<b>Lead beneficiary</b> <sup>10</sup>	1 - AIT
<b>Work package title</b>	Project Management and Coordination		
<b>Start month</b>	1	<b>End month</b>	48

### Objectives

- O1.1 Efficient project management ensuring progress in line with the budget and the schedule
- O1.2 Risk management and overall strategic project guidance
- O1.3 Project administration, fulfilling all contractual and reporting obligations
- O1.4 Building and maintaining effective communication channels within the consortium

### Description of work and role of partners

#### **WP2 - Project Management and Coordination** [Months: 1-48]

**AIT, ACTECO, AIMPLAS, AVECOM, TUC, IMGGE, IBET, LIT, LOGOPLASTE, MicroLife, NTUA, TCD**  
Description of work

The work includes four tasks: T1.1, T1.2 and T1.3. All of the partners including the three international partners SDU, BIT and CAS will contribute to each of tasks T1.1, T1.2 and T1.3. SDU, BIT and CAS are linked to AIT for WP 1.

#### Task 1.1: Project Management

Role of partners: AIT will be the task leader and will be responsible for all activities. All WP leaders participate in the Steering Committee. All partners will participate in appointing members of the General Assembly and experts for quality control.

This task covers the management of the project, including the following activities:

- Project planning and supervising including detailed work plan preparation and progress monitoring;
- Day-to-day project coordination by the Management Team as described in Section 3.2;
- Establishment of the General Assembly to provide the overall strategic direction, take major decisions, manage the risks and handle the IPR issues, as described in Section 3.2;
- Establishment of the Steering Committee – including monthly video conferences as described in Section 3.2;
- Quality control - involving peer review by at least one internal expert for each deliverable;
- Maintain relations with the European Commission and with other key players;
- Development, regular update and implementation of the risk management strategy; and
- Intellectual Property Rights management.

#### Task 1.2: General Assembly meetings and consortium communication

Role of partners: AIT will be the task leader and will be responsible for all activities, chairing all meetings and developing all meeting minutes. All partners will participate in this task, hosting meetings, participating in all meetings and telephone conferences as required.

As described in Section 3.2, the main governing body of the project is the General Assembly, which consists of one representative from each project partner. The General Assembly will meet once a year to chart progress, identify potential problems and their solutions, and to refine the the work-programme for each partner for the subsequent 12 month period. Upon signing of the Grant Agreement, a kick-off meeting will be organised in Athlone, Ireland (at the AIT premises) and then three further general assembly meetings will follow, hosted by different partners.

In between the general assembly meetings, there will be technical meetings bringing together smaller teams cooperating on specific tasks. In addition, regular communication will be maintained through:

- Frequent e-mail exchanges and bilateral telephone calls;
- Monthly telephone conferences between the coordinator and the leaders of the tasks that are running to monitor project progress and deal with possible problems; and
- Use of an internet-based communication platform and file repository (such as Google Drive), which will be set-up by AIT to provide the consortium members with a platform for communication, which is well documented, quick and structured, allowing all partners to readily follow the progress of all tasks in the project

#### Task 1.3: Administrative and financial management

Role of partners: AIT will be the task leader and will be responsible for all activities. All partners will participate and provide their contributions to the project reporting.

This task encompasses all financial and administrative activities, including:

- Follow-up on any issues relating to the Consortium Agreement (CA) and Grant Agreement (GA);

- Representation of the beneficiaries at the European Commission, acting as the intermediary for any communication between the Commission and any beneficiary;
- Receiving the financial contribution to the project on behalf of the beneficiaries and administering its allocation, in accordance with the Grant Agreement, the Consortium Agreement and any decisions taken by the General Assembly and keeping all relevant records;
- Ensure that the partners are aware of and fulfil all their Grant Agreement responsibilities and reporting duties;
- Stay in close contact with the EC representatives informing them about the progress of the project; and
- Implement the periodic and final technical and financial reporting to the EC according to the GA requirements

#### Task 1.4 Technical management

Management of technical activities will ensure project technical outputs are inline with the project objectives. Annual updates will be carried out including risk management strategy implementation

### Participation per Partner

Partner number and short name	WP2 effort
1 - AIT	39.00
2 - ACTECO	0.50
3 - AIMPLAS	3.00
4 - AVECOM	2.00
5 - TUC	2.00
6 - IMGGE	2.00
7 - IBET	4.00
8 - LIT	2.00
9 - LOGOPLASTE	1.00
10 - MicroLife	2.00
11 - NTUA	2.00
12 - TCD	2.00
<b>Total</b>	<b>61.50</b>

### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D2.1	Internet based communication platform and repository	1 - AIT	Websites, patents filling, etc.	Confidential, only for members of the consortium (including the Commission Services)	2
D2.2	Technical management	1 - AIT	data sets, microdata, etc	Confidential, only for members of the consortium (including the Commission Services)	3
D2.3	Report with all Executive Board meetings	1 - AIT	Report	Confidential, only for members of the	48

### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
				consortium (including the Commission Services)	

### Description of deliverables

D1.1: Internet based communication platform and repository (Month 2)

D1.2: Technical management with annual updates including risk management plan (Month 3 and ongoing)

D2.1 : Internet based communication platform and repository [2]

An internet-based communication platform and file repository (such as Google Drive), which will be set-up by AIT to provide the consortium members with a platform for communication, which is well documented, quick and structured, allowing all partners to readily follow the progress of all tasks in the project

D2.2 : Technical management [3]

Technical management to ensure project technical outputs are inline with the project objectives. Annual updates will be carried out including risk management strategy implementation

D2.3 : Report with all Executive Board meetings [48]

Reports will be provided on the monthly Executive Board video-conferences meetings which will give updates on the progress of each work package.

### Schedule of relevant Milestones

Milestone number <sup>18</sup>	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS1	Completion of all reporting	1 - AIT	48	All project plans delivered and reporting complete

<b>Work package number</b> <sup>9</sup>	WP3	<b>Lead beneficiary</b> <sup>10</sup>	12 - TCD
<b>Work package title</b>	Pre-Treatment Processes for Plastic Bio-Degradation		
<b>Start month</b>	2	<b>End month</b>	48

### Objectives

The overall aim of this work package to develop and optimise the pre-treatment technologies to enhance the microbial degradation of plastic waste by using various microorganisms (WP3) for individual/mixed non-biodegradable and bio-degradable plastics including:

O2.1 Characterisation Identification of plastic waste feedstock for pre-treatments;

O2.2 Designing novel pre-treatment processes to improve the accessibility of plastic waste for microbial degradation;

O2.3 Testing the effect of various pre-treatment methods to generate carbon sources suitable for valorisation;

O2.4 Monitoring the physical, chemical, thermal, and mechanical properties of plastic waste to monitor the efficiency of pre-treatment processes;

O2.5 Designing the separation process for the recovery of available carbon source for valorisation from pre-treatment process;

O2.6 To formulate compatible polymer blends using pre-treated and microbial degraded plastic waste as a compatibiliser and evaluation of suitability polymer blends for 3D Printing; and

O2.7 Ensure the commercial scalability and environmental sustainability of pre-treatment processes

### Description of work and role of partners

#### **WP3 - Pre-Treatment Processes for Plastic Bio-Degradation** [Months: 2-48]

**TCD, AIT, ACTECO, AIMPLAS, TUC, IBET, LOGOPLASTE**

This WP is led by TCD and the contributing partners are AIT, ACT, AIM, TUC and IBET. The international partners do not contribute to WP 2.

This work package presents the first of three actions in the solution to reduce the impact of non-biodegradable polymers spread in the environment. The hydrophobicity, high MW, chemical and structural composition of most petroleum based plastics hinders their biodegradation. A number of physical, chemical, mechanical, and photo/thermal oxidation approaches will be evaluated for enhancing their biodegradation and increasing amenability to biological degradation. This WP will also evaluate, develop, and integrate the outputs of WP6 and WP7 for applications including food packaging and filaments for 3D printing.

**Task 2.1: Formulating and analysing of plastic waste feedstock** (Task Leader: TCD. Task Contributor: ACT)

Post-consumer plastic waste is generally a heterogeneous mixture, in terms of both polymer types and its composition. Waste collection systems across various regions and countries produce different types of plastic waste. Initial work will be focused on formulating and identifying mixed plastic waste feedstock for BioICEP project based on the information available from commercial recycling companies. Three types of plastic waste mixes will be created alongside of individual targeted polymers.

1. Mix containing non-biodegradable polymers: Polyethylene 29% (LDPE, LLDPE (17%), HDPE (12%) ), PolyPropylene (19%), Polyvinyl chloride (12%), Polystyrene (8%), PET (6%), Polyurethane (7%), and additives and processing aids (17%);

2. Mix containing mixture of non-degradable and bio-degradable polymers: Polyethylene 29% ( LDPE, LLDPE (17%), HDPE (12%) ), PolyPropylene (19%), Polyvinyl chloride (12%), Polystyrene (8%), PET (6%), Polyurethane (7%), PLA (2%), PHA (1%), starch (1%) and additives and processing aids (13%); and

3. Mix Containing biodegradable polymers: PLA (50%), PHB (10%), Starch (10%), PBS (5%), PCL (15%) and processing additives (10%)

The mixes will be shredded into micro pieces or ground into powders to create feedstock for pre-treatment process. The physical and chemical properties of the feedstock will be evaluated alongside of mixed plastic waste collected from commercial plastic recycling companies.

**Task 2.2: Evaluation of pre-treatment technologies** (Task Leader: TCD. Task Contributor: AIT, AIM). This task will exploit pre-treatment processes to enhance the bio-degradation of individual and mixed plastic waste in WP3 using selected microbial consortia. To maximize the efficiency of pre-treatment processes, a combination of pre-treatment processes will be evaluated. In order to extract low molecular weight compounds/oligomers produced during pre-treatment process, TCD has recently developed a method using ultrasonication and supercritical carbon dioxide with

green solvents and has initiated a patent application. In order to optimise the pre-treatment processes, samples will be characterized for their thermal, mechanical, chemical, and physical properties. The oligomers and modified polymer compounds that are obtained during the pre-treatment processes will be evaluated (Task 2.3) as a compatibiliser to create compatible polymer blends and adaptability of these blends for 3D printing (FDM process) in designing the circular polymer composites from plastic waste.

Task 2.2.1: Ultra sonication: (Task Leader: TCD. Task Contributor: CUT) Ultrasonication is a very effective mechanical pre-treatment method based on cavitation to modify and degrade the polymers. The pre-treatment step involves dispersion of plastic waste in sustainable solvents such as water, cyrene, ethanol, vegetable oils, and surfactants followed by ultrasonication. Various process parameters such as temperature, pressure, flow rate, and ultrasonic frequency (up to 1500 Hz) will be optimized in a batch process to maximize the effect of pre-treatment for polymer degradation. At the end of ultrasonication process, solid samples are separated by filtration for further characterization and to provide the samples for WP3. From the collected liquid fraction, the dissolved organics will be separated by precipitation or solvent evaporation. A continuous ultrasonication process will be evaluated with optimized process parameters to provide samples (50-100g) for WP3. The efficiency of the pre-treatment process will be evaluated by deriving the specific energy (Ws/gr) values for the optimized processes. This work will run in parallel to other WP2 tasks and feed in to WP3 and WP6.

Task 2.2.2: Microwave thermal degradation: (Task Leader: AIM. Task Contributors: TCD and AIT). Both individual and mixed plastic waste will be subjected microwave thermal degradation in a batch reactor in the presence of high microwave absorber materials such as carbon. A design of experiments will be performed to optimise various process parameters such as temperature range, microwave power, exposure time, and catalyst nature to produce the substrate for microbial degradation. The amount of gases, oils, and chars produced will be quantified and analysed (Task 2.4) and the efficiency of the pre-treatment process is evaluated. With optimised process conditions, samples of 50g scale will be produced and supplied to the partners (WP3).

Task 2.2.3: Supercritical Carbon dioxide (ScCO<sub>2</sub>) assisted Depolymerisation: (Task Leader: AIM. Task Contributor: TCD) In order to provide substrate for microbial degradation (WP3) and valorization (WP6), de-polymerization assisted with ScCO<sub>2</sub> will be performed for individual selected polymers (such as PET, PS, PE, PU, PLA, PBS, and Starch) followed by mixed plastic waste. This approach will have the dual advantage of facilitating the fresh surface for depolymerizing agents to penetrate through the plastic and improving the process by increasing the solubility of ScCO<sub>2</sub> in the plastic waste. Initial experiments will be carried out using HAAKE MiniLab extruder. Experiments will be performed taking into account different temperatures profiles, residence times, feed rates and feeding design, polymer types, ScCO<sub>2</sub> pressure, ScCO<sub>2</sub> flow, and ScCO<sub>2</sub> addition time to optimize various process parameters. Addition degradation promoters such as ethylene glycol will be evaluated to promote the depolymerisation during the extrusion process. Similarly, various process parameters will be optimised for mixed plastic waste to obtain pre-treated feedstock for microbial degradation (WP2) and Valorization (WP6). Based on the preliminary data, two types of individual plastics and two types of mix plastic waste substrates will be processed on a large-scale extruder to produce kg-level samples for characterization, microbial degradation, and valorization. To avoid the emission of hazardous substances, activated carbon-based filters will be utilised. The products obtained consisting in mixtures of oligomers and, in some cases, monomers will be characterized in subtask 2.4.2.

Task 2.2.4: Reactive extrusion (Task Leader: AIM. Task Contributor: TCD). The reactive extrusion process will be applied to pre-treat the individual and mixed plastic waste samples. Reactive extrusion (REX) is of industrial importance because it is solvent-free, continuous, scalable, and highly flexible for a range of plastics to modify their physical and chemical properties by depolymerisation. In this task, the depolymerization of PET, LDPE, polystyrene, polyethylene and polyurethanes will be investigated separately by reactive extrusion. Furthermore, selected bio-based polymers such as PLA, PBS, PHA and starch will be also studied by REX to enhance the microbial biodegradation. In order to enhance the rate of the degradation, the use of free radical initiators and catalysts will be considered. A study of the effect temperature, residence time, pressure and extrusion screw speed, and configuration will be conducted. The initial trials will be conducted using HAAKE™ MiniLab extruder or Brabender lab mixer and once the best conditions in terms of overall efficiency have been identified, the process will be scaled up in a pilot scale twin-screw extruder to produce Kg-quantity samples.

Task 2.2.5: UV-degradation (Task Leader: AIT. Task Contributor: TCD) UV-assisted photo degradation will be carried out with selected individual polymers on 10gr scale using UV-B radiation lamp in an enclosed chamber. The exposure of plastics to UV light will trigger the photooxidation leading the chain scission of polymer backbone and produce carbonyl (-C=O) and vinyl (-CH<sub>2</sub>=CH<sub>2</sub>) type compounds which are more susceptible microbial degradation. Various process parameters such as exposure time, wavelength, and % moisture content will be evaluated on the degradation of individual polymers and polymer mixes. In order to accelerate the photo oxidation process, catalytic amounts of photosensitive compounds such as organic peroxides, Zinc Oxide, MoS<sub>2</sub>, Boron nitride, Phosphorus nitride, Zirconium



Selenide will be blended with plastic sample before exposure to UV light. The degradation products will be characterised ( Task 2.3) for further optimisation.

Task 2.2.6: Blending with natural polymers and Additives (Task Leader: TCD. Task Contributors: AIT and AIM). In order to modify and enhance the microbial degradation of plastic waste by thermo-oxidative degradation, blends of plastics waste with biodegradable/natural polymers, pro-oxidants and unsaturated polymers will be formulated. Master batches of will be prepared with degradable polymers such as polylactic acid, starch, polycaprolactone, polyhydroxyalkonate, polybutylene succinate along with prooxidants such as cobalt, manganese, and copper stearates and unsaturated butadiene and styrene type of co-polymers. Blends of plastic waste with various amounts (2-10wt %)-master batches will be prepared using lab scale Brabender mixer. In order to obtain blends with maximum accessibility for microbial degradation, various process parameters such as processing temperature, torque, processing time, and the volume of master batch will be optimised. Samples with maximum degradability will supplied for WP3 partners for microbial degradation.

Task 2.3: Evaluation: Identification of pre-treated plastic compounds as a compatibiliser for polymer blends suitable 3D printing (Task Leader: TCD. Task Contributor: AIT)

Oligomers, functional low MW polymers with unsaturation, produced from pre-treatment process can act as good compatiabiliser for non-compatible polymer blends. Polymer blends using the combinations of polymers such as HDPE, Nylon, PEBAX, PLA, PHB, and PCL will be prepared using lab scale Brabender melt mixer. The fully miscible compositions characterised by mechanical properties will be used to produce filaments for 3D printing using a pilot scale digital micrometer controlled filament production line (AIT). Using a 3DGence FDM printer, dog bone specimens will be printed using the filaments obtained from polymer blends for evaluation of mechanical properties. In order to evaluate the circularity of these polymer blends, the 3D objects created from the compatabilised blends will be subjected to pre-treatment process (WP2) and microbial degradation (WP3).

Task 2.4 Characterization of the pre-treated plastics and degradation products isolated (Task Leader: AIT. Task Contributors: TCD and ACT). Characterisation of thermal, chemical, mechanical, physical, and molecular properties of the pre-treated samples will be carried out to understand and optimise the efficiency of the pre-treatment processes. The products produced in sub-tasks 2.2.1- 2.2.6 will be characterized employing several analytical techniques. To determine the crystallinity of pre-treated plastic waste, X-ray diffraction and Differential Calorimetry (DSC) will be performed. The change in the chemical structure is evaluated by infrared spectroscopy (FTIR), liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy (NMR) analysis. DSC analysis to determine the melting temperature of the oligomeric/degraded products, gel permeation chromatography (GPC) to determine their MW, polydispersity index, and rheological tests will be utilised to measure the behaviour in melt state of the oligomers obtained during the pre-treatment process. The analysis of possible hazardous contaminants resulted from the pre-treatment process will be analysed by gas-liquid chromatography analysis (GC). The residual compounds present in the pre-treated samples will be analysed by XPS, SEM-EDX and ICP-MS analysis. Static contact angle analysis will be used to verify the hydrophilic and hydrophobic nature of pre-treated samples to assess the accessibility of substrates for microorganism.

Task 2.5: Optimisation of the Pre-treatment process (Task Leader: TCD. Task Contributor: AIT and AIM). Both individual plastics and mixed plastic substrates will be subject to selected pre-treatment processes. This task aims to optimise the TWO selected pre-treatment processes, either stand-alone or a combination of pre-treatment processes to develop a scalable, higher efficiency platform for a predictable substrate for microbial degradation and valorisation. The optimised process will consist of pre-treatment process combined with either ultra sonication or ScCO<sub>2</sub> extraction processes to produce more suitable substrate for valorisation. A continuous ultra sonication and separation process will be evaluated as a pre-treatment process to extract valaorisable carbon source. This work will run in parallel to other WP2 tasks and will feed into WP6 for valorisation.

#### Participation per Partner

Partner number and short name	WP3 effort
1 - AIT	24.00
2 - ACTECO	13.00
3 - AIMPLAS	38.00
5 - TUC	9.00
7 - IBET	1.00

Partner number and short name	WP3 effort
9 - LOGOPLASTE	4.00
12 - TCD	38.00
<b>Total</b>	<b>127.00</b>

#### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D3.1	Plastic waste partner supply report	12 - TCD	Report	Confidential, only for members of the consortium (including the Commission Services)	6
D3.2	New plastic waste pre-treatment process report	12 - TCD	Report	Confidential, only for members of the consortium (including the Commission Services)	24
D3.3	Report on degraded plastic waste based compatibilisers	12 - TCD	Report	Confidential, only for members of the consortium (including the Commission Services)	48

#### Description of deliverables

D2.1 Plastic waste characterisation report and supply plan to partners (Month 6, TCD)  
D2.2 New pre-treatment process for plastic waste report (Month 24)  
D2.3 Technical report on pre-treated and microbial degraded plastic as a compatibiliser (Month 36, TCD)

D3.1 : Plastic waste partner supply report [6]  
A report will be prepared on categorised post-consumer mixed plastic waste feedstock and the supply of characterised shredded/ground feedstocks to consortium partners.

D3.2 : New plastic waste pre-treatment process report [24]  
A report evaluating combination pre-treatment processes for the extraction low molecular weight compounds/ oligomers will be provided.

D3.3 : Report on degraded plastic waste based compatibilisers [48]  
A report will be provided on the identification of pre-treated plastic low molecular weight output molecules and compounds as compatibilisers for polymer blends suitable for 3D printing

#### Schedule of relevant Milestones

Milestone number <sup>18</sup>	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS2	Plastic waste feedstock	12 - TCD	12	Categorised, characterised and prepared post use plastic

**Schedule of relevant Milestones**

<b>Milestone number<sup>18</sup></b>	<b>Milestone title</b>	<b>Lead beneficiary</b>	<b>Due Date (in months)</b>	<b>Means of verification</b>
				waste feedstock supply to partners
MS3	Optimised Pretreatment process	12 - TCD	24	Optimised and integrated pretreatment process for mixed plastics

<b>Work package number</b> <sup>9</sup>	WP4	<b>Lead beneficiary</b> <sup>10</sup>	6 - IMGGE
<b>Work package title</b>	Development of Enzymatic and Biocatalytic Solutions for Single and Mixed Plastic Degradation		
<b>Start month</b>	2	<b>End month</b>	46

## Objectives

### Objectives

- O3.1. Screen existing enzymes (existing within consortium and reported in the literature) for their ability to depolymerise various types of plastics through standard plate assays using homogenised selected recalcitrant plastic substrates (PET, LDPE, PS, HDPE and PU), bio-based polymer substrates (PLA, PBS, starch and PHB), and their mixtures.
- O3.2. Synthesis of novel substrates for the detection of plastics degradation enzymatic activities.
- O3.3. Setup novel enzymatic assays for screening enzymes with plastic degrading activities and surface analysis of plastics for the elucidation of the enzymatic attack mechanisms.
- O3.4. Identification of novel enzymatic activities through genome sequencing and MS protein identification.
- O3.5. Protein engineering biocatalyst improvement of up to three target biocatalysts for the efficient degradation of certain recalcitrant plastics.
- O3.6. Design of a microbial platform with coupled degradation-synthetic capabilities.

## Description of work and role of partners

### WP4 - Development of Enzymatic and Biocatalytic Solutions for Single and Mixed Plastic Degradation

[Months: 2-46]

**IMGGE**, AVECOM, IBET, MicroLife, NTUA

IMGGE leads WP3 and AVE, IBET, MLS, CAS and SDU contribute to this WP. The international partners SDU and CAS are linked to AIT and NTUA for the work in WP 3. On the 8 tasks the two international partners contributions are as follows:

SDCU contributes to T3.1 –T3.4, T3.6, T3.7.

CAS contributes to T3.3, T3.4, T3.6 and T3.7

The purpose of WP3 is to develop and form plastics enzymatic or biocatalytic treatments to deliver monomers/monomer mixtures for valorisation. WP3 defines and tests a cocktail of enzymes that can be used at various stages of pre-treatment and after pre-treatment of mixed plastic waste. Novel and innovative screening strategies for biodegradation of plastic materials including a high throughput screening strategy and development of novel high performance strain biosensors for expedited advancement of single and mixed plastics degradation will be demonstrated. Biocatalysts will be improved using engineering approaches, formulations, and generation of platforms with high degrading as well as biopolymer synthetic performance that will be supplied for valorisation in WP6.

### Description of work:

The purpose of WP3 is to develop and form plastics enzymatic or biocatalytic treatments to deliver monomers/monomer mixtures for valorisation. WP3 defines and tests a cocktail of enzymes that can be used at various stages of pre-treatment and after pre-treatment of mixed plastic waste. Novel and innovative screening strategies for biodegradation of plastic materials including a high throughput screening strategy and development of novel high performance strain biosensors for expedited advancement of single and mixed plastics degradation will be demonstrated. Biocatalysts will be improved using engineering approaches, formulations, and generation of platforms with high degrading as well as biopolymer synthetic performance that will be supplied for valorisation in WP6.

### Tasks

T3.1: Production and chromatographic purification of selected enzymes on 20 to 50 mg scale for the screening experiments (Task Leader IMGGE. Task Contributors: NTUA, and SDU): Approximately 100 enzymes will be selected amongst those reported in the literature for their ability to depolymerize each of the selected plastic materials and those identified amongst members of consortium (see Annex ). Enzymes will be evaluated in agarose plate assay using emulsified polymeric substrates , NTUA has cutinases , several esterases, laccases , peroxidases etc. that will be utilised. IMGGE also has collection of biocatalysts mostly from Streptomyces strains including laccases, cutinases, and other esterases.

Recombinant enzymes can be purified from crude extracellular (Pichia pastoris host) or intracellular (Escherichia coli) fractions using Immobilized Metal Affinity Chromatography (IMAC).

T3.2: Synthesis of model compounds for assaying plastics degradation enzymatic activities (Task Leader:IMGGE. Task Contributor: SDU):

T3.2.1 A library of 13 compounds will be synthesised using standard organocatalysis procedures on 10-20 g scale (Fig. 9). These substrates will be provided to WP4 and WP5 for screening purposes, and also to WP6 in preparation for bioproduct development.

Figure 9 Suggested model compounds to be synthesized and used in enzymatic screens

T3.2.2 The transcription regulatory protein-based whole-cell biosensors of the degradation products (e.g. terephthalic acid) will be developed. Directed evolution will be performed to engineer the ligand binding pocket of the transcription regulatory proteins, and the mutants responsive to the target compound (e.g. terephthalic acid) will be selected. In the whole-cell biosensors developed using the regulatory protein mutants, the expression of fluorescent protein is regulated by the concentration of the surrounding degradation products. Several small-molecule biosensors using this strategy have been developed by CAS group.

T3.3: Establishment of novel assays for screening microbial enzymes for plastics degradation potential (plate and liquid assays using model compounds and their defined mixtures) and surface degradation analysis (Task Leader: IMGGE. Task Contributors: NTUA, SDU, AIT, TCD, and CUT):

T3.3.1 96-well plate assays (200  $\mu$ L well-volume) will be developed using model substrates for each of selected plastic materials for high-throughput standardized screening with either substrate depletion or product formation monitoring protocol and spectral analysis (HPLC and/or GC coupled with MS). This approach will quantitatively monitor the enzymatic reactions and assess the synergistic activity of the selected enzymes using single and defined mixtures of substrates in reaction with enzyme mixes. This approach may provide a method to reduce the total amount of enzyme loading required to achieve a similar extent of hydrolysis. The degree of synergy (DS) will be calculated using standard equation:  $DS = \frac{c1+c2}{c1+c2+2}$ , where  $c1+c2$  is the monomer concentration produced during hydrolysis when two enzymes are added;  $c1$  and  $c2$  are monomer concentration produced, respectively, when respective hydrolyses are added individually during hydrolysis.

T3.3.2 The whole-cell biosensors described in T.4.2.2. are able to report the degradation product concentrations via cell fluorescence, which can be efficiently screened using Fluorescence Activated Cell Sorting (FACS), agar plates or 96-well plates. Under the same substrate concentration, the higher concentration of degradation products will indicate the improvement of degrading enzyme activities.

T3.3.3 Surface properties of the pre-treated plastics will be measured using very sensitive analytical methods as Ultraviolet Photoelectron Spectroscopy in combination with metastable (Helium atoms) Induced Electron spectroscopy (UPS/MIES) in order to characterize the outermost surface groups and so to elucidate the mechanism of the enzymatic attack on the surface. This task will be performed in cooperation with the group in NTUA, who will provide pre-treated samples for AIT, TCD and CUT after enzymatic attack for certain time(s).

T3.4 Purification and biochemical characterization of novel enzymatic activities (Task Leader: IMGGE. Task Contributors: NTUA and SDU)

Once the best performing strains from the novel pool of strains are identified from WP4 and WP5, the new enzymatic activities will be pursued using genome analysis of up to five strains, construction of genome libraries, and by chromatographic protein isolation and MS protein identification analysis. High-throughput screening will be performed using standard plate assays or the biosensors of the degradation product. This combined approach would allow for improved chances to identify novel degrading enzymes in reasonable time-frame.

T3.5 Immobilization of target enzymatic activities (Biocatalyst stabilization) (Task Leader: IMGGE. Task Contributor: NTUA)

T3.5.1 Enzyme immobilization. Immobilisation of enzymes effectively increases their stability, reusability, and reduces the overall cost and environmental impact of the biotechnological process. Solid supports like xerogels, sand, and clay are widely used at scale. Another approach that does not involve utilization of carrier materials will be used, namely cross-linking of enzyme aggregates (CLEAs) such as magnetic CLEAs, porous-CLEAs and combi-CLEAs by interconnecting enzyme molecules via multiple bonds using crosslinking agents such as glutaraldehyde. This approach has been widely utilized for the stabilization and immobilization of enzymes used at industrial scale (such as lignocellulose treatment). The high catalyst density and microporous assembly of CLEAs guarantee high catalyst activity, that together with their long shelf life, operational stability, and reusability, provide a cost-efficient alternative. An easier and cheaper alternative is the encapsulation of enzymes in alginate gels. Alginate is a non-toxic, biodegradable material suitable for support of enzymes. Entrapment in alginate gels can be combined with other immobilization techniques like CLEAs to increase stability. Furthermore, in order to increase the stability of entrapped enzymes their activation with glutaraldehyde can take place before encapsulation.

**T3.5.2 Whole-cell approaches & microbial cell surface display** Whole-cell biocatalysts provide unique advantages and have been widely used for the efficient biosynthesis of value-added fine and bulk chemicals and they can be rationally designed. The advantages include efficiency, catalyst cost, and easier downstream processing. More importantly, the presence of the protective cellular envelope stabilizes the enzymes and enables applications under harsh conditions that are otherwise inhibiting to enzyme coupled the presence of all necessary co-factors. Optimized biocatalysts for each of the targeted plastic materials will be assessed in whole-cell *E. coli* formulations, using various concentration of catalysts (OD600 of 5-100) and several initial concentration of substrate, to arrive at an optimized strategy for the mixed plastic substrates.

Microbial cell surface display strategy, i.e. accord of enzymes to be exhibited on the surface of cells by fusing the proteins of interest with the anchoring motifs, will be carried out using *E. coli* and/or *Pichia* as hosts. Various anchors for expression in *E. coli*, such as AIDA-I, FadL and OmpC will be used, while for *P. pastoris* Flo9, Pir1 and Sed1p could be used for surface display of target enzymes.

**T3.6 Evolution of target enzymatic activities for the efficient degradation of defined plastics waste materials** (Task Leader: IMGGE. Task Contributors: SDU and CAS). Directed evolution methodology is a powerful tool for enzyme improvement, and it has been successfully utilised in industrial enzyme development to improve temperature tolerance, introduce pH tolerance, enhance specific activity, and alter substrate specificity. The PETase obtained from a PET-consuming bacterium *Ideonella sakaiensis* was reported to exhibit superb hydrolytic activity and substrate preference towards PET. Site-directed mutagenesis has been performed, leading to improved activity of the enzyme.

Although it can be applied to multigene pathways and gene networks, BioICEP will focus on the laboratory evolution of single genes that have identified as promising for the depolymerization of selected plastic materials. We will evolve PHA-depolymerize and cutinase that can efficiently depolymerize PHB to accept mcl-PHA as substrate using random and site-specific (depending on the availability of protein sequence and structure) mutagenesis (standard protocols using kits i.e. GeneMorph® II Random Mutagenesis kit or Phusion Site-Directed Mutagenesis Kit GeneArt™ Site-Directed Mutagenesis PLUS System). This would be undertaken due to the fact that a recent study has identified PHA as not readily biodegradable under home composting conditions. In addition, highly active PETase or other plastics degrading enzymes identified in this project will be used for directed evolution to further improve their activities in order to develop highly efficient enzyme cocktails for plastics degradation. The mutagenesis libraries will be screened using established high-throughput assays or the biosensors of the degradation products.

**T3.7 Construction of a microbial platform for ‘microbial-cell factory’ using Systems Biocatalysis approach** (Task Leader: IMGGE. Task Contributors: NTUA and SDU). Systems Biocatalysis is a new approach consisting of organizing enzymes in vitro to generate an artificial metabolism for synthetic purposes. The strategy of this key new platform involves the analysis of enzymatic systems in vivo, as well as their assembly in vitro into novel synthetic metabolic pathways that enable the production of value-added chemicals from carbon-based feedstock. Therefore, the introduction of monomer degradation capabilities into PHA (*Pseudomonas putida* or *Ralstonia eutropha* H16), rhamnolipid (*P. aeruginosa*), and nanocellulose (*Komagataibacter mendelliensis*) producing strains or PHA- and nanocellulose-biosynthetic clusters into plastic monomers degrading strains using a CRISPR-Cas9 strategy is planned. The procedure for efficient genome editing has been developed for PHB producing *R. eutropha* using an electroporation-based CRISPR-Cas9 technique (Xiong et al. 2018).

**T3.8 Validation of best performing Biocatalytic Solutions for degradation of pre-treated mixed plastic waste material** (Task Leader: AVE). Biocatalytic Solutions (including enzymes, enzyme cocktails, stabilized biocatalysts) will be evaluated based on the predetermined set of criteria to determine the selection that would be utilised on pre-treated plastic mixed waste material on the larger scale.

#### Participation per Partner

Partner number and short name	WP4 effort
4 - AVECOM	3.00
6 - IMGGE	90.00
7 - IBET	3.00
10 - MicroLife	8.00
11 - NTUA	19.00

Partner number and short name	WP4 effort
<b>Total</b>	123.00

#### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D4.1	Report on the synthesis of model compounds	6 - IMGGE	Report	Confidential, only for members of the consortium (including the Commission Services)	12
D4.2	Demonstration of new accelerated Screening	6 - IMGGE	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	24
D4.3	Report on mechanism of enzymatic and/or microbial attack	6 - IMGGE	Report	Confidential, only for members of the consortium (including the Commission Services)	24
D4.4	Report on discovery of at least four novel enzymatic activities	6 - IMGGE	Report	Confidential, only for members of the consortium (including the Commission Services)	30
D4.5	Report on Enzymatic Immobilization	6 - IMGGE	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D4.6	Biocatalyst improvement by directed evolution	11 - NTUA	Report	Confidential, only for members of the consortium (including the Commission Services)	40
D4.7	Construction of the microbial cell factory	6 - IMGGE	Demonstrator	Confidential, only for members of the consortium (including the Commission Services)	46

#### Description of deliverables

D3.1 Report on the synthesis of model compounds that mimics different plastics (Month 12)  
D3.2 Demonstrate new accelerated screening by novel in situ biosensors which flag high performing strains (Month 24)  
D3.3 Report on mechanism of enzymatic and/or microbial attack on the pre-treated plastics (Month 24)  
D3.4 Discovery of at least four novel enzymatic activities capable of degrading plastics (Month 30)  
D3.5 Report on the enzyme or whole cell immobilization of target biocatalysts (Month 36)



D3.6 Report on the improved of target biocatalysts through directed evolution (Month 40)
D3.7 Construction of the microbial cell factory for the conversion of plastics waste into valuable products (Month 46)
D4.1 : Report on the synthesis of model compounds [12] A report on the synthesis of model compounds for assaying plastics degradation enzymatic activities will be provided
D4.2 : Demonstration of new accelerated Screening [24] Demonstration of novel assay(s) established for screening microbial enzymes for plastics degradation potential (plate and liquid assays using model compounds and their defined mixtures) and surface degradation analysis
D4.3 : Report on mechanism of enzymatic and/or microbial attack [24] Report on mechanism of enzymatic and/or microbial attack including genome analysis of enzymatic activities and construction of genome library
D4.4 : Report on discovery of at least four novel enzymatic activities [30] A report on the discovery of at least four novel enzymatic activities based on the laboratory evolution of single genes identified as promising for the depolymerization of selected plastic materials.
D4.5 : Report on Enzymatic Immobilization [36] A report on the establishment of enzymatic immobilisation including entrapment/encapsulation of enzyme aggregates or whole cell surface display of target enzymes.
D4.6 : Biocatalyst improvement by directed evolution [40] Report on biocatalysts improvements by directed evolution including the formulation of enzymatic cocktails with high polymer degrading performance.
D4.7 : Construction of the microbial cell factory [46] Demonstration of a microbial platform for 'microbial-cell factory' using Systems Biocatalysis approach

#### Schedule of relevant Milestones

Milestone number <sup>18</sup>	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS4	Model substrates for plastic degradation assays	6 - IMGGE	6	Model plastic substrates for assaying enzyme & microbial activities
MS5	Novel degrading enzyme cocktails	6 - IMGGE	24	Novel enzymatic cocktails with high degradation activities
MS6	Platform for plastic waste bioconversion	6 - IMGGE	46	Microbial platform for simultaneous degradation & synthesis



<b>Work package number</b> <sup>9</sup>	WP5	<b>Lead beneficiary</b> <sup>10</sup>	8 - LIT
<b>Work package title</b>	Establishment of a catalogue of high performance microbial strains for plastic degradation and bioplastic production		
<b>Start month</b>	2	<b>End month</b>	38

### Objectives

O4.1 Screen existing microbial biobanks within the work partners for their ability to degrade various types of plastics.  
O4.2. Screen new sources of plastic degrading microbes including waste plastics from Serbian and Chinese polluted sites.  
O4.3. Generate and test strains with boosted hydrolytic activity and ability to degrade mechano-biochemical treated plastics providing feedback to WP 2to further optimise the pre-treatment methods.  
O4.4. Identify the best enzyme producers from all screens for the breakdown of individual and/or mixed plastics to feed into WP3.  
O4.5. Quantitative analysis of breakdown potential and dynamics (efficiency, speed) for each of the selected plastics.  
O4.6. Screen identified degraders (in O4.1 and O4.2) for PHB, rhamnolipid, and nanocellulose production potential.  
O4.7. Based on the feedback from WP3 and WP5, optimisation of strain selection to form the best individual plastic and mixed plastic degrading consortia strains to re-feed into WP5 and identify the best strains from all screens to form consortia, which will feed into WP5 and WP6.

### Description of work and role of partners

#### **WP5 - Establishment of a catalogue of high performance microbial strains for plastic degradation and bioplastic production** [Months: 2-38]

**LIT**, AIT, TUC, IMGGE, MicroLife, NTUA

LIT leads WP4 and AIT, MLS, AVE, IMGGE, NTUA, TUC, BIT, CAS and SDU contribute to the seven tasks. The international partners SDU, CAS and BIT are linked to AIT and LIT for the work in WP 4.

The international partner contributions to the tasks are as follows:

SDU: Contributing to T4.1-T4.2 and T4.4-T4.7

CAS: Contributing to T4.6

BIT: Contributing to T4.1-T4.5 and-T4.7

In this work package, biodiscovery screening of consortium partner's existing biobank and newly isolated strains, for the identification of selected efficient plastic bio-degraders. This screen will be undertaken initially with individual type of plastics and then the chosen organisms will be grown in consortia for degradation of mixed plastics, mimicking environmental plastic population. All work partners already have existing microbial biobanks, which they can test for their ability to break down of the selected plastics immediately after launch of the project. Later, sources of plastic waste will be used to isolate new strains using conventional approach and also by iCHIP method, which will then be screened for their ability to breakdown the selected plastics. The identified degraders will be characterised by all partners for their ability to degrade targeted plastics after pre-treatment, depolymerisation enzyme activities, and provide relevant information to WP2, WP5 and WP6. The best strains from all screens will used to identify and isolate novel enzymatic activities (WP3) and to create defined consortia in WP5, which can breakdown mixed plastic waste. 'Designer strains' will be generated to boost plastic hydrolysing capacities based on microbial host platforms such as *Streptomyces lividans* and *Pichia pastoris*.

Also the identified degraders will be screened by all partners for PHB, rhamnolipid, and nanocellulose production potential and recommend to WP6. Sharing of identified strains, their growth conditions for consortia optimisation will take place between consortium partners after signing of appropriate Material Transfer Agreement (MTA).

#### Description of work and role of participants:

In this work package, biodiscovery screening of consortium partner's existing biobank and newly isolated strains, for the identification of selected efficient plastic bio-degraders. This screen will be undertaken initially with individual type of plastics and then the chosen organisms will be grown in consortia for degradation of mixed plastics, mimicking environmental plastic population. All work partners already have existing microbial biobanks, which they can test for their ability to break down of the selected plastics immediately after launch of the project. Later, sources of plastic waste will be used to isolate new strains using conventional approach and also by iCHIP method, which will then be screened for their ability to breakdown the selected plastics. The identified degraders will be characterised by all partners for

their ability to degrade targeted plastics after pre-treatment, depolymerisation enzyme activities, and provide relevant information to WP2, WP5 and WP6. The best strains from all screens will be used to identify and isolate novel enzymatic activities (WP3) and to create defined consortia in WP5, which can breakdown mixed plastic waste. 'Designer strains' will be generated to boost plastic hydrolysing capacities based on microbial host platforms such as *Streptomyces lividans* and *Pichia pastoris*.

Also the identified degraders will be screened by all partners for PHB, rhamnolipid, and nanocellulose production potential and recommend to WP6. Sharing of identified strains, their growth conditions for consortia optimisation will take place between consortium partners after signing of appropriate Material Transfer Agreement (MTA).

#### Tasks:

T4.1. Biodiscovery screen of (existing and new) microbial biobanks: (Task Leader: LIT. Contributor: MLS, AVE, IMGGE, NTUA and BIT) Following the standard technique for screening of plastic-degrading microorganisms, which results in clear-halo zone on agar (high-grade agar with reduced alternate carbon source or agarose) plates, will be used by all partners for identifying plastic-degrading potential of their biobank strains. Selected recalcitrant plastic substrates (PET, LDPE, Polystyrene, HDPE and PU) and bio-based polymer substrates will be used either emulsified or dispersed in the medium as a fine powder as sole carbon source as described earlier.

T4.2 Isolation of new microbes and biobank enrichment: (Task Leader: LIT. Contributor: MLS, AVE, IMGGE, NTUA and BIT). Samples from waste plastics from landfills and marine habitats will be collected and brought to the laboratory for isolation using conventional plating technique. Biological material adhered to the plastic substrates will be removed by gentle scraping, vortex and/or mild-sonication as appropriate in sterile water (10 ml). After serial dilution (10<sup>-5</sup>) of each sample, the last three dilutions will be plated on nutrient agar for isolation of bacterial and fungal strains. If some field samples are coloured particularly greenish/blackish, will be plated on modified BB/BG-11 (for fresh water) and/or ASN-III (for marine) agar medium for the isolation of photosynthetic microalgae/cyanobacteria. Following the iCHIP method using an empty rack from the Matrix TallTip Extended Length Pipette Tip box, we will isolate the bacterial and fungal strains on neutral agar with standard mixed plastics and nutrient agar supplemented with homogenised plastics to ensure that we obtain isolates capable of mineralization of plastic materials, as well as the ones with the potential to depolymerize them. The last three dilutions as above of each sample will be mixed with molten agar for the diffusion chambers in iCHIP and sealed with 0.03- $\mu$ m-pore-size polycarbonate membranes to each side of the rack, and then incubate the iCHIPs to the corresponding natural habitat for 1-3 months before final laboratory isolation.

T4.3 Construction of a single microbial platform for boosting plastics degradation capacity (Task Leader: IMGGE. Contributor: NTUA and BIT)

Microbial production of value-added chemicals from plastic waste as carbon source (Wierckx et al. 2015) is a sustainable alternative to chemical synthesis, however to improve product titer, yield, and selectivity, the pathways engineered into microbes must be optimized. Therefore, strains to express and co-express multiple hydrolases will be developed, using alternative hosts:

T4.3.1 – *Streptomyces lividans* and/or *Streptomyces albus* - as Gram-positive soil bacteria with a well-studied and differentiated morphology. They are important candidates for the production of heterologous proteins for several reasons, including their efficient secretion mechanism, capability of excreting large amounts of proteins, an exceptionally low protease activity, and their robust growth. All this is industrially very useful due to the reduction of downstream processing and good product stability. We will generate several strains to encompass single or combinations of best performing hydrolases in *Streptomyces* host using antibiotic marker-free system previously described. The total absence of antibiotic resistance genes makes this system a powerful tool for using *Streptomyces* spp. as a host to produce proteins at the industrial level. This approach responds well with the safety concerns and legal requirements surrounding the increased use of antibiotic resistance genes in recombinant protein production. *S. lividans*  $\Delta$ TA-Tox strain will be used for this purpose, according to the described procedure 43.

T4.3.2 – *Pichia pastoris* is a GRAS methylotrophic yeast that is frequently used as a protein expression system thanks to the many advantages it shows: growth to very high cell densities, strong and tightly regulated promoters, and options to produce gram amounts of recombinant protein per litre of culture both intracellularly and in secretory fashion. In addition, the methylotrophic yeast *P. pastoris* expression system, as a eukaryotic expression system, has been a favourite system for expressing heterologous proteins due to its many advantages, such as protein processing, protein folding, and post-translational modification.

T4.4. Liquid media cultivation with standard plastics: (Task Leader: LIT. Contributor: MLS, AVE, IMGGE, NTUA and BIT). This task will cultivate the pre-identified plastic degrading organisms from T4.1, T4.2 and T4.3 in appropriate minimal media with standard plastics (single and in mixes). This test will be performed in flasks containing minimal media and the plastics and inoculated with selected microbes from above tasks. Following the growth period, the culture supernatant (cell-free extracts) and spent medium (source for extracellular enzymes) will be collected and tested for depolymerase enzyme activities. The plastics after microbial degradation will be collected and analysed as part of T4.6.

T4.5. Liquid media cultivation with pre-treated plastics: (Task Leader: LIT Contributor: MLS, AVE, IMGGE, NTUA and BIT). Test the identified plastic degraders from liquid cultivation in T4.4 for their ability to degrade targeted plastics which have been pre-treated as part of WP2. This test will be performed in flasks containing minimal media containing pre-treated plastic and inoculated with selected microbes from above T4.4 Following the growth period, the culture supernatant (cell-free extracts) and spent medium (source for extracellular enzymes) will be collected and tested for depolymerase enzyme activities. The plastic after microbial degradation will be collected and analysed as part of T4.6.

T4.6. Quantitative/qualitative analysis of plastic breakdown potential and dynamics: (Task Leader: LIT. Contributor: AIT, MLS, AVE, IMGGE, NTUA, CAS and SDU). Plastic subjected to microbial degradation in T4.4 and T4.5 will be analysed in this task. The reduction in weight of biologically treated plastics will be recorded by digital balance which could accurately measure up to 0.01 mg. The thickness of plastics will be measured using digital micrometre capable of measuring up to 0.001 mm. Weight loss efficiency will be calculated using following equation,

$$\text{Weight reduction (\%)} = [(W_0 - W_t) \times 100] / W_0$$

Where,  $W_0$  is the initial weight of plastic (g),  $W_t$  is the weight of plastic (g) at time 't' (days). Rate constants for plastic degradation will be determined using the relation given below:

$$-\ln (W_t/W_0) = kt$$

Where  $W_0$  is the initial weight of plastic (g),  $W_t$  is the weight of plastic (g) at time 't' (days) after microbial inoculation. A plot of  $\ln W_t/W_0$  versus 't' yields a slope equal to 'k' which is rate constant.

The chemical fingerprint of the biodegraded plastics will be assessed by attenuated total reflectance (ATR)-FTIR spectroscopy used at a frequency range of 4500–400  $\text{cm}^{-1}$  for analysis. To investigate the chemical modifications of the plastics induced by the microbial growth, the IR spectra of the plastics before and after inoculation with microbes will be compared. For these analyses, 10 randomly chosen spots will be assayed in three replicates of treated and untreated plastic. The surface morphology of the plastics before and after microbial biodegradation will be examined by Scanning Electron Microscopy (SEM).

T4.7. Identification of potential PHB, rhamnolipid, and nanocellulose producers: (Task Leader: LIT. Contributor: MLS, AVE, IMGGE, NTUA and BIT). Screen pre-identified degraders from T4.1 and T4.2 as potential producers for PHB, rhamnolipid and/or nanocellulose production after cultivating these strains on breakdown plastic products (styrene, terephthalic acid, isocyanates, ethylene glycol, polyol, lactic acid, 3-hydroxybutyrate, 3-hydroxyoctanoate, and glucose) (in broth or on plates as appropriate). Nile red or Nile blue A staining plate assay method will be used for the identification of PHA/PHB producers. In brief, plates will be prepared with neutral agar, breakdown plastic products and Nile red or Nile blue A, and after incubation with the test organism, plates will be exposed to ultraviolet light (312 nm) to visualise stained intracellular PHA/PHB granules. PHB production and content will further be confirmed through liquid cultivation of the suspected positive organisms. Epifluorescence microscopy using Nile-red staining for rapid identification of short-chain-length and medium-chain-length PHBs will be used as described earlier. From liquid cultivation, 5-15 mg dried biomass will be used for GC-MS analysis for precise information on the content and type of PHB produced. Blue agar plate assay method will be used for the identification of rhamnolipid producers. In brief, plates will be prepared with neutral agar, breakdown plastic products, cetyl-trimethyl-ammonium bromide and methylene blue, and after incubation with the test organism, appearance of dark blue halo zone around the culture will be considered positive for rhamnolipids. Buffered Schramm & Hestrin's (BSH) agar plate assay method will be used for the identification of nanocellulose producers. In brief, pre-identified degraders will be cultured on BSH medium (carbon source replaced with breakdown plastic products) statically for 2 weeks. Culture broth showing pellicles formation will then be spread onto BSH agar plates and incubated again for a week. The colonies with milk-white and swollen appearance will be isolated as potential nanocellulose producers.

#### Participation per Partner

Partner number and short name	WP5 effort
1 - AIT	6.00
5 - TUC	12.00
6 - IMGGE	6.00
8 - LIT	56.00
10 - MicroLife	8.00
11 - NTUA	8.00

Partner number and short name	WP5 effort
<b>Total</b>	96.00

#### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D5.1	Identification of efficient microbial plastic degraders	8 - LIT	Report	Confidential, only for members of the consortium (including the Commission Services)	20
D5.2	Generation of novel boosted degradation capacity strains	8 - LIT	data sets, microdata, etc	Confidential, only for members of the consortium (including the Commission Services)	24
D5.3	Liquid cultivation conditions for pre-identified degraders	8 - LIT	Report	Confidential, only for members of the consortium (including the Commission Services)	28
D5.4	Characterisation of depolymerase enzyme activities	6 - IMGGE	Report	Confidential, only for members of the consortium (including the Commission Services)	33
D5.5	Quantitative/ qualitative analysis of plastic breakdown characterisation	8 - LIT	Report	Confidential, only for members of the consortium (including the Commission Services)	34
D5.6	PHB, rhamnolipids and nanocellulose producers	8 - LIT	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D5.7	Recommended strains for WP3, WP5 and WP6	8 - LIT	Report	Confidential, only for members of the consortium (including the Commission Services)	36

#### Description of deliverables

D4.1. Identification of efficient microbial degraders of plastics from existing and new biobanks (Month 20).  
D4.2. Generation of novel strains with boosted plastic degradation capacities (Month 24).  
D4.3 Establishment of liquid cultivation conditions for pre-identified degraders on standard & pre-treated plastics (Mth 28)  
D4.4 Characterisation of depolymerase enzyme activities after liquid cultivation (Month 33).  
D4.5. Quantitative and qualitative characterisation of plastic breakdown potential and dynamics (Month 34).

D4.6 Identification of PHB, rhamnolipids and nanocellulose producers (Month 36).

D4.7 Consolidated identification and recommendation of the best microbes and their growth conditions to support WP4, WP5 and WP6 for various up-scaling optimisations. (Month 36).

D5.1 : Identification of efficient microbial plastic degraders [20]

Identification of efficient microbial plastic degraders using biodiscovery screening of consortium partner's existing biobank, newly isolated strains from collected waste plastics

D5.2 : Generation of novel boosted degradation capacity strains [24]

Generation of novel boosted plastic hydrolysing capacity based on microbial host platforms

D5.3 : Liquid cultivation conditions for pre-identified degraders [28]

Liquid cultivation conditions for pre-identified plastic degrading microbes in appropriate minimal media with standard, mechano-green chemical pretreated and biocatalysed plastics (single and in mixes).

D5.4 : Characterisation of depolymerase enzyme activities [33]

Characterisation of depolymerase enzyme activities using quantitatively monitoring of the enzymatic reactions and assessment of the synergistic activity of the selected enzymes using single and defined mixtures of substrates in reaction.

D5.5 : Quantitative/qualitative analysis of plastic breakdown characterisation [34]

A report will be provided on the quantitative/qualitative analysis of plastic breakdown potential and dynamics including weight loss efficiency and chemical assessment of the biodegraded plastics.

D5.6 : PHB, rhamnolipids and nanocellulose producers [36]

A report on pre-identified degraders as potential producers of PHB, rhamnolipid and/or nanocellulose.

D5.7 : Recommended strains for WP3, WP5 and WP6 [36]

A report will be provided recommending the best strains from all screens for the identification of and isolation of novel enzymatic activities (WP3) and to create defined consortia in WP5, which can breakdown mixed plastic waste. Also the identified degraders screened for PHB, rhamnolipid, and nanocellulose production potential will be recommended to WP6

#### Schedule of relevant Milestones

Milestone number <sup>18</sup>	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS7	Catalogue of best microbial degraders	8 - LIT	36	Catalogue of Identified best microbial biodegraders and value-added biomolecule producers

<b>Work package number</b> <sup>9</sup>	WP6	<b>Lead beneficiary</b> <sup>10</sup>	10 - MicroLife
<b>Work package title</b>	Establishment of high performance microbial consortia for plastic degradation and bioplastic production		
<b>Start month</b>	4	<b>End month</b>	46

### Objectives

O5.1 Formation of stable microbial communities suitable for surface modifications of recalcitrant plastics).

O5.2 Formation of stable natural and synthetic communities based on established plastic degrading strains recommended from WP4.

O5.3 Development of new enriched selected communities with increased plastic degradation capacities.

O5.4 Establishment of communities with high resilience to the contaminants and chemicals present in pre-treated plastics and mixed plastics (sourced from WP2).

O5.5 Establishment of the microbial community platform with coupled degradation-synthetic capabilities.

O5.6 Monitoring of the microbial community composition and performance during various stages of bioprocesses.

O5.7 Validation of best-performing microbial communities against pre-treated mixed plastic waste in bioreactors.

### Description of work and role of partners

#### **WP6 - Establishment of high performance microbial consortia for plastic degradation and bioplastic production** [Months: 4-46]

**MicroLife**, AIT, AVECOM, IMGGE, IBET, LIT, NTUA

MLS leads WP5 and LIT, AVE, IMGGE, NTUA, AIT, BIT, CAS and SDU contribute to the seven tasks. The international partners SDU and CAS and BIT are linked to AIT for the work in WP 5.

The international partner contributions to the tasks are as follows:

SDU: Contributing to T5.1-T5.4, T5.6 and T5.7

CAS: Contributing to T5.1 and T5.2

BIT: Contributing to T5.1-T5.4, T5.6 and T5.7

Physiochemical properties of synthetic plastics present obstacles for their enzymatic degradation especially when they are in the form of mixed plastic waste materials, therefore WP5 will develop stable microbial communities or defined mixes of microbial strains with improved performance in comparison to whole cells or single strains, to be applied at various stages of biological degradation, as well as at valorisation stages. This will be achieved in close collaboration with WP3 and WP4 which develop biocatalysts and single-strains for plastic degradation and synthesis of biopolymers and rhamnolipids. We will also use exploration and functional screening of novel microbial diversity by isolating new microbial communities from the contaminated sites such as landfills. Therefore, established communities will be validated in both mixed plastic degradation, as well as in valorisation experiments (WP6). Standard formulation techniques for development of mixed microbial inoculants will be applied, as well as principles of synthetic biology in attempts to engineer microbial community for efficient mixed plastic biotechnological recycling process. Communities will be monitored during the process using proprietary bioinformatics pipeline.

#### Description of work and role of participants:

Physiochemical properties of synthetic plastics present obstacles for their enzymatic degradation especially when they are in the form of mixed plastic waste materials, therefore WP5 will develop stable microbial communities or defined mixes of microbial strains with improved performance in comparison to whole cells or single strains, to be applied at various stages of biological degradation, as well as at valorisation stages. This will be achieved in close collaboration with WP3 and WP4 which develop biocatalysts and single-strains for plastic degradation and synthesis of biopolymers and rhamnolipids. We will also use exploration and functional screening of novel microbial diversity by isolating new microbial communities from the contaminated sites such as landfills. Therefore, established communities will be validated in both mixed plastic degradation, as well as in valorisation experiments (WP6). Standard formulation techniques for development of mixed microbial inoculants will be applied, as well as principles of synthetic biology in attempts to engineer microbial community for efficient mixed plastic biotechnological recycling process. Communities will be monitored during the process using proprietary bioinformatics pipeline.

#### Objectives

O5.1 Formation of stable microbial communities suitable for surface modifications of recalcitrant plastics).



O5.2 Formation of stable natural and synthetic communities based on established plastic degrading strains recommended from WP4.

O5.3 Development of new enriched selected communities with increased plastic degradation capacities.

O5.4 Establishment of communities with high resilience to the contaminants and chemicals present in pre-treated plastics and mixed plastics (sourced from WP2).

O5.5 Establishment of the microbial community platform with coupled degradation-synthetic capabilities.

O5.6 Monitoring of the microbial community composition and performance during various stages of bioprocesses.

O5.7 Validation of best-performing microbial communities against pre-treated mixed plastic waste in bioreactors).

#### Tasks:

T5.1 Establishment of the stable mixed community of bacteria and fungi suitable for biological treatment of mixed plastic waste coming from WP2. (Task Leader: MLS. Contributor: LIT, AVE, IMGGE, NTUA, CAS and SDU).

T5.1.1. – Microbial consortia to deal with highly recalcitrant plastics. This microbial consortium will consist of both bacterial and fungal species from BioICEP partners' biobanks and from the strains reported in the literature for the ability to make recalcitrant plastics (especially ones with carbon-carbon backbones such as PE and PS) more amenable to enzymatic depolymerisations. These organisms will be expressing a number of laccases, manganese peroxidases, lignin peroxidases as well as hydroquinone peroxidases. Synergistic effect of microbial communities on various single plastic materials has been reported in the literature. Novel microbial communities from petroleum contaminated sites will be isolated and functionally screened against single and mixed plastic materials using standard methodologies established in WP3 and WP4.

T5.1.2. – Microbial consortia to deal with possibly toxic materials. Impact of pre-treatment on microbial communities. In WP2 several pre-treatment methods will be tested to make plastics more available for microbial and enzymatic degradation. During the pre-treatment processes chemicals may be released or formed that negatively impact microbial and/or enzymatic performance. An example of this the release of antimony trioxide, a catalysis used for polymerisation, from PET which is present in trace amounts. Thus to the consortium from T5.1.1 metal tolerant and strains capable of dealing with possibly inhibitory factors released during pretreatments of mixed plastic waste (WP2) will be included. Plastics from the different pre-treatment methods will be used to test their impact on the degrading community stability and its ability to degrade the plastics. This process will be iterative and combined with the work from WP2 to select optimal pre-treatment processes that both make the plastic available for microbial breakdown but have limited impact on the microbial community or enzyme activity.

T5.2 Forming synthetic communities using established plastic degrading strains. (Task Leader: MLS. Contributor: LIT, AVE, IMGGE, NTUA, CAS and SDU). After determining optimal plastic breakdown potential of existing strains and communities and of newly discovered communities from WP4, synthetic communities will be formed. As it is highly likely that different types of plastics will be degraded by different microbes, synthetic communities will be established from the species selected from WP4 using the principles set out by Johns et al. to establish synthetic communities. Synthetic communities will be analyzed for community stability using taxonomic sequencing as well as its potential to breakdown mixed plastic waste streams into plastic monomer constituents.

T5.3 Forming and enrichment of relevant, natural communities. (MLS, LIT, AVE, IMGGE, NTUA and SDU). This will be carried out to increase their plastic degradation potential. Relevant existing communities which contain plastic degrading microbes will probably contain these organisms at low densities. By enriching them on plastic model compounds (mostly plastic dimers) to be used as a carbon source the density of the plastic degrading microbes will be increased and species that do not contribute to the degradation process will be lost yielding a relevant and efficient degrading community. Communities already present at the project partners but also newly discovered communities will be subjected to enrichment processes using model compounds selected and tested in WP3. Subsequently these enriched communities will be tested on their breakdown capacity of mixed plastic waste streams.

T5.4 Taxonomic identification of microbial consortia members and sequencing of (meta)genomes. (Task Leader: MLS. Contributor: AVE, IMGGE, NTUA and SDU). After the selection of optimal consortia in tasks 3.1 and 3.2 the best performing consortia will be taxonomically identified using 16S ribosomal DNA sequencing using either Illumina MySeq (V3-V4 region) or using Oxford Nanopore (ONT) MinION sequencing. Simultaneously, we will use functional sequencing on both the communities and single species to identify the enzymes involved in plastic degradation using ONT MinION in combination with custom bioinformatic pipelines developed by MLS. Information on species and enzymes will be used in WP3 and WP5 to further optimize the plastic degradation consortia.

T5.5 Identification of plastic transformation and breakdown products from degradation by microbial consortia. (Task Leader: MLS. Contributor: LIT, AVE, IMGGE, NTUA, TCD and AIT). After optimal breakdown consortia have been established in previous tasks, we will investigate which breakdown products are generated from the selected types of plastic and how these breakdown products can feed into subsequent fermentation processes to generate new products

(WP6). Examples of breakdown products can be plastic monomers such as terephthalic acid from PET or oligomers which consist of short chains of the respective plastic monomers. Ideally the selected breakdown processes would not mineralize plastics to CO<sub>2</sub> and H<sub>2</sub>O so the breakdown products can be used as a carbon source to ferment into new products. Plastic breakdown products will be analyzed using GC-MS and/or LC-MS.

T5.6 Establishment of defined microbial consortia for the simultaneous plastic degradation and product (PHB, rhamnolipids and nanocellulose) formation. (Task Leader: MLS. Contributor: LIT, AVE, IMGGE, NTUA and SDU). From WP3 and WP4 strains with the capability to utilise plastic monomers and oligomers as a sole source of carbon and energy, as well as to contain biosynthetic pathways for PHB or biosurfactant synthesis will be identified or generated. Successful utilisation of defined and natural consortia for PHA production from mixed carbon sources have previously been described. Establishment of the successful consortia to simultaneously release monomers and use them for value-added product formation will be utilised using previously described approaches and principles of synthetic biology .

T5.7: Protocol for optimal breakdown parameters. (Task Leader: MLS. Contributor: TCD, AIT, LIT, AVE, IMGGE, NTUA and SDU) In this task, information from the previous tasks in WP5 and from WP4 will be combined to select optimal breakdown parameters (pretreatment, community composition, etc) for mixed waste plastic degradation. These parameters will be combined with information about optimal fermentation parameters from WP6 to design a pilot process that handles the whole plastic microbial recycling chain from plastic degradation into fermentation of new polymers.

#### Participation per Partner

Partner number and short name	WP6 effort
1 - AIT	6.00
4 - AVECOM	12.00
6 - IMGGE	18.00
7 - IBET	6.00
8 - LIT	10.00
10 - MicroLife	18.00
11 - NTUA	7.00
<b>Total</b>	<b>77.00</b>

#### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D6.1	Report on enrichment of existing microbial communities	10 - MicroLife	Report	Confidential, only for members of the consortium (including the Commission Services)	18
D6.2	Report on synthetic community vs individual performance	10 - MicroLife	Report	Confidential, only for members of the consortium (including the Commission Services)	24
D6.3	Identification of degrading enzymes	10 - MicroLife	Report	Confidential, only for members of the consortium (including	30



### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
	within best performing communities			the Commission Services)	
D6.4	Optimized synthetic and natural microbial communities	10 - MicroLife	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D6.5	Information on plastic breakdown products for WP6	1 - AIT	Report	Confidential, only for members of the consortium (including the Commission Services)	42

### Description of deliverables

D5.1. Report on plastic degradation enrichment properties and potential of existing microbial communities. (Month 18)

D5.2. Report on synthetic community vs individual microbe performance for plastic breakdown based on combined data from WP3 and WP5. (Month 24)

D5.3. Report on minimal, optimized community composition and plastic degrading enzymes present in these communities based on (meta)genomic DNA sequencing. (Month 30)

D5.4. Establishment of optimized synthetic and enriched natural plastic degradation microbial communities which will breakdown at least 20% of relevant, non-biodegradable plastics. (Month 36)

D5.5. Information on plastic breakdown products to be fed as carbon sources into the fermentation processes developed by WP6. (Month 42)

D6.1 : Report on enrichment of existing microbial communities [18]  
Report on enrichment of existing microbial communities for increased mixed plastic waste stream breakdown capacity.

D6.2 : Report on synthetic community vs individual performance [24]  
Report on individual strain performance verses performance of synthetic community established from the species selected from WP4 in the breakdown mixed plastic waste streams

D6.3 : Identification of degrading enzymes within best performing communities [30]  
A report on taxonomically identified degrading enzymes within best performing consortia will be provided

D6.4 : Optimized synthetic and natural microbial communities [36]  
Report will be provided on optimized synthetic and natural microbial communities for plastic degradation and breakdown products based on information from the previous tasks in WP5 and from WP4 its implementation in the selection of optimal breakdown parameters (pretreatment, community composition, etc) for mixed waste plastic degradation

D6.5 : Information on plastic breakdown products for WP6 [42]  
A report on the chemical analysis of breakdown products generated from the selected types of plastic and how these breakdown products can feed into subsequent fermentation processes to generate new products will be provided

**Schedule of relevant Milestones**

<b>Milestone number<sup>18</sup></b>	<b>Milestone title</b>	<b>Lead beneficiary</b>	<b>Due Date (in months)</b>	<b>Means of verification</b>
MS8	Optimal performing Microbial Consortia	10 - MicroLife	46	Optimal performing Microbial Consortia degrading greater than 20% of mixed plastics

<b>Work package number</b> <sup>9</sup>	WP7	<b>Lead beneficiary</b> <sup>10</sup>	7 - IBET
<b>Work package title</b>	Bioprocess Development for Production of Value-added Biopolymer Products		
<b>Start month</b>	8	<b>End month</b>	48

### Objectives

O6.1. Development of bioprocesses for the production of PHB with distinct monomer composition and functional properties, using waste synthetic plastics' monomers as feedstock.

O6.2. Development of bioprocesses for the production of nanocellulose using waste synthetic plastics' monomers as feedstock.

O6.3. Development of bioprocesses for the production of different types of rhamnolipids, using waste synthetic plastics' constituent molecules and monomers as feedstock.

O6.4. Process optimization by online monitoring and metabolic modelling.

O6.5. Protocols for the preparation of bioproduct with properties with high performance characteristics for application in end use products as achieved using a feedback process with chemical, mechanical and thermal analysis and WP 3, WP4 and WP5.

### Description of work and role of partners

#### **WP7 - Bioprocess Development for Production of Value-added Biopolymer Products** [Months: 8-48]

**IBET**, AIT, AIMPLAS, AVECOM, IMGGE, LOGOPLASTE, MicroLife, TCD

IBET leads WP6 and IMGGE, NTUA, TCD, ACT, SDU, LIT, MLS, AIT, AIM, AVE contribute to the tasks. The international partner SDU is linked to AIT and IBET for the work in WP 6.

The international partner contributions to the task are as follows:

SDU: Contributing to T6.1-T6.2

The goal of WP6 is to convert the constituent molecules and monomers obtained from waste synthetic plastics degradation into value-added microbial products, namely, PHB/PHA, nanocellulose, and rhamnolipids. Different microbial consortia and enzyme cocktails, developed and demonstrated in WP3, W4 and WP5 for their ability to synthesize one or more of the envisaged products will be used to develop and optimize bioprocesses for their high yield production in bioreactor experiments. The bioproduction as well as the downstream process will be optimized at laboratory scale using advanced monitoring techniques and metabolic modelling. Extensive testing, processing and analysis of the bioproducts will be carried out with comparative testing with respect to current on the market equivalent products in applications such as food packaging. This will facilitate a feedback process to allow the optimisation of the fermentation process for enhancement of the bioproducts and will enable improved processing quality and integrity. Data for a preliminary cost assessment for each product will be provided to WP7.

#### Tasks:

T6.1. Bioprocesses optimization (Laboratory scale) (Task Leader: IEBT. Contributor: IMGGE, NTUA, TCD, ACT, SDU, LIT, MLS, AIM, AVE). In this task, several bacteria screened in WP4 and WP5 and selected for their ability to produce PHBs, PHAs, nanocellulose and/or rhamnolipids will be tested for their ability to utilize waste synthetic plastics' monomers for cell growth and products synthesis. The selected strains (up to 4) will be cultivated in 2 L bioreactors under controlled conditions of temperature, pH, dissolved oxygen concentration, aeration, and stirring rate, aiming at defining optimal cultivation conditions in order to get high productivities in a reproducible way. Optimization of the operational conditions, monomer concentration, medium composition and feeding strategies, will be performed, assisted by online bioreactor monitoring using spectroscopy (FT-NIR and Raman) and chemometrics. Samples will be periodically collected from the bioreactors during the fermentations and will be analysed off-line for biomass, PHB, PHA, nanocellulose, and rhamnolipids production. NIR and Raman spectra will be acquired at-line for the same samples to guarantee that the spectra correspond to the exact off-line analytical characterization. Principal component analysis (PCA) models will be developed with the obtained spectra to identify outliers and to characterize the fermentation batches without the use of further analytical information. The results from the off-line routine analyses will be used with the corresponding spectra for the development and validation of partial least squares (PLS) models for the in-line prediction of biomass, PHB, PHA, nanocellulose, and rhamnolipids content. The produced products will be recovered from the broth and characterized to evaluate their characteristics that will be considered as criteria for strain selection.

T6.2. Process validation at 10 L reactor (Laboratory scale) (Task Leader: IEBT. Contributor: AVE, MLS, IMGGE and SDU). Three strains, each producing one of the envisaged microbial products, will be cultivated in 10 L bioreactors

to validate the cultivation conditions and define the cultivation protocol for further scaling-up studies. The bioreactors will be monitored online using the spectroscopy-based models developed in Task 6.1 aiming at acquiring real-time data for process optimization and control. Guidelines for process design will be established for the implementation of the processes at pilot scale. and data for a preliminary cost assessment will be provided in collaboration with AVE in WP7.

**T6.3. Metabolic modelling and bioprocess design (Task Leader: IEBT. Contributor:IMGGE, NTUA)**

Metabolic models will be developed for selected production strains in order to better understand metabolic bottlenecks and to optimize process operation targeting maximum productivity and maximum yield. The metabolic models will be essentially based on the collection of well-established biochemical transformations including those involved in the metabolization of plastic monomers while avoiding hard assumptions on kinetic mechanisms. Methods of systematic metabolic pathway analysis will be employed to fully characterize the metabolic functionality of the selected strains. In a second stage, the established models will be employed for process design. First the models will be used to design culture media composition based on rational design principles that maximize carbon flow through desired (optimal) conversion pathways. Second the designed formulations will be assessed experimentally coordinated with task 6.1 (small scale). Finally, the metabolic models will be adopted to optimize bioreactor control also coordinated with tasks 6.1 and 6.2. More specifically, the metabolic models will be used to optimize the feeding strategies of key compounds, such as the plastic monomers feeding rate along time, as well as other critical medium components that deplete along time.

**T6.4. Development and optimization of downstream procedures for products' recovery (Task Leader: IEBT. Contributor: TCD, AVE and AIT).** The products obtained in task 6.2 will be recovered from the broth using green approaches. Extraction of PHB/PHA will be performed by using non-hazardous solvents. Nanocellulose will be purified by alkali treatment. Rhamnolipids will be recovered and purified using non-hazardous solvents and environmentally friendly procedures. The degree of purification for each product will be evaluated. For each product, after purification, analysis of the potential contaminants will be carried out and potential impacts will be determined by ecotoxicity assessment at AIT.

**T6.5. Chemical, mechanical, and thermal characterization and analysis. (Task Leader: IEBT Contributor: AIT and AIM)** Information on the bioproduct chemical structure, MW and MW distribution, identification of the chemical composition and crystallinity which are an important parameter for processing and establishing end use applications will be carried out using suites of chemical, mechanical, and thermal analysis. Techniques will include Fourier Transform Infrared Spectroscopy (FTIR), Gel Permeation Chromatography (GPC), Gas Chromatography Mass Spectrometry (GPC-MS), X-ray diffraction, Goniometry and Scanning Electron Microscopy (SEM). High performance liquid chromatography (HPLC) will be used to establish the purity and identify components in the biopolymers. Thermal analysis will be carried out using Differential scanning calorimetry (DSC), Thermogravimetric analysis (TGA), Melt flow index (MFI). Mechanical Characterisation: Mechanical analysis will include rheometry for determination of the storage modulus, loss modulus, viscosity, and shear strength of the biopolymers. Dynamic Tensile testing, Compression testing and 3-point bend flexural testing and Impact resistance will be performed. The results of this testing will feedback into earlier tasks (T6.2 and T6.3) allowing the bioprocess to be refined optimised for the generation of high quality bioproducts with properties appropriate to applications in market segments such as the food and pharmaceutical industry.

**Participation per Partner**

Partner number and short name	WP7 effort
1 - AIT	8.00
3 - AIMPLAS	14.00
4 - AVECOM	4.00
6 - IMGGE	56.00
7 - IBET	12.00
9 - LOGOPLASTE	23.00
10 - MicroLife	2.00
12 - TCD	7.00
<b>Total</b>	<b>126.00</b>

### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D7.1	Report on the best strains and process operation conditions	7 - IBET	Report	Confidential, only for members of the consortium (including the Commission Services)	20
D7.2	Report on the metabolic model and monitoring	7 - IBET	Report	Confidential, only for members of the consortium (including the Commission Services)	38
D7.3	Report on optimised PHBs, nanocellulose and rhamnolipids	1 - AIT	Report	Confidential, only for members of the consortium (including the Commission Services)	44
D7.4	Protocols on the optimized conditions for downstream process	7 - IBET	Report	Confidential, only for members of the consortium (including the Commission Services)	48

### Description of deliverables

D6.1. Report on the best strains and process operation conditions to produce the target products (Month 20).  
D6.2. Report on the metabolic model and monitoring for process optimization (Month 38).  
D6.3. Samples and protocols for the production of PHBs, nanocellulose, and rhamnolipids with high performance mechanical and chemical properties suitable for processing for high value end use products. (Month 44).  
D6.4. Protocols and report on the optimized conditions for downstream process (Month 48).

D7.1 : Report on the best strains and process operation conditions [20]  
A report will be prepared on the bioprocessing of strains selected for their ability to produce PHBs, PHAs, nanocellulose and/or rhamnolipids utilizing waste synthetic plastics' monomers as feedstock for cell growth and products synthesis

D7.2 : Report on the metabolic model and monitoring [38]  
A report will be prepared on inline bioreactor monitoring and metabolic models developed to better understand metabolic bottlenecks and to optimize process operation targeting maximum productivity and maximum yield.

D7.3 : Report on optimised PHBs, nanocellulose and rhamnolipids [44]  
A report on the chemical, mechanical, and thermal characterization and analysis of optimised PHBs, nanocellulose and rhamnolipids will be prepared

D7.4 : Protocols on the optimized conditions for downstream process [48]  
Protocols will be prepared on the optimized conditions for downstream process for the preparation of bioproduct with properties with high performance characteristics will be developed using a feedback process involving chemical, mechanical and thermal analysis

### Schedule of relevant Milestones

Milestone number <sup>18</sup>	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS9	Established bioproduct bioprocess	7 - IBET	48	Established bioprocess for the production of high performance PHB, nanocellulose and rhamnolipid bioproducts for high need market segments within the food and pharmaceutical industry

<b>Work package number</b> <sup>9</sup>	WP8	<b>Lead beneficiary</b> <sup>10</sup>	4 - AVECOM
<b>Work package title</b>	Establishment BioICEP Pilot plant for mixed plastics degradation and bioproduct production		
<b>Start month</b>	12	<b>End month</b>	48

### Objectives

- O7.1. Establishment of integrated automatized small scale BioICEP pilot plant.  
O7.2. Operation of the BioICEP pilot plant for 20%+ mixed plastics degradation.  
O7.3. Small scale pilot production of high performance PHB and nanocellulose for applications such as food packaging and rhamnolipids for pharmaceutical applications.  
O7.4. Life Cycle Analysis demonstrating BioICEP low environmental impact and favourable position compared with current end emergent competitor technologies.  
O7.5. BioICEP Business model presenting the go-to-market potential and market projections.

### Description of work and role of partners

**WP8 - Establishment BioICEP Pilot plant for mixed plastics degradation and bioproduct production** [Months: 12-48]

**AVECOM**, AIT, ACTECO, AIMPLAS, IMGGE, IBET, TCD

AVE leads WP7 and IBET, IMGGE, TCD, ACT, LIT, MLS, AIT, AIM, BIT and SDU contribute to the tasks. The international partners SDU and BIT are linked to AIT for the work in WP 7.

The international partner contributions to the task are as follows:

SDU: Contributing to T7.2-T7.4

BIT : Contributing to T7.2-T7.4 and T7.6

AVE will implement the BioICEP process developed in WP2-6 at pilot scale (50-100 L). A pilot plant will be constructed and process control and automatization will be implemented. This integrated pilot system will include a modular biocatalytic and microbial pretreated plastics degradation bioreactor, biomass separation, and bioproduct fermentation operated in accordance with the parameters developed in WPs 2-6. The generation of PHB/rhamnolipid/nanocellulose bioproduct will be optimised and fully characterised to ensure their potential for end of use applications. The pilot will be designed for the operation with non-genetically modified microorganisms and the setup will be constructed at AVE's tech hall (Ghent, Belgium). Additionally, aife cycle analysis will be carried out in conjunction to TCD in order to establish the environmental impacts of each stage of the technology.

Tasks:

T7.1. Construction of Pilot reactor according to specification input from WP2, 3, 4 and 5. (Task leader: AVE. Contributor: IEBT, IMGGE, NTUA, MLS). AVE will receive all the specifications required to design and construct a pilot reactor setup (not a full demonstration, therefore pilot volumes will be in the range of 50-100L). The optimum specification should determine important reactor characteristics such as the optimum pH, temperature, aeration (strict aerobic, micro-aerobic or strict anaerobic), agitation, solid residence time (SRL), hydraulic residence time, and continuous or batch-wise feeding (related to this is the harvesting method: continuous effluent efflux, batch wise efflux of homogeneous effluent or batch wise sedimentation and efflux of fluid effluent).

T7.2. Pilot reactor Setup. (Task leader: AVE. Contributor: IEBT, IMGGE, NTUA, SDU, MLS. AIT, AIM). The reactor setup will be based on a modular operation which will allow the regulation of biocatalytic and microbial pretreated plastics degradation processes in accordance with the parameters developed in WP 3 and 5. These will be selected in order to provide the most suitable depolymerised mixed plastic feedstock for the fermentation of the required bioproduct according to the protocols developed in WP6. The reactor setup will consist of three operation units: (i) biocatalytic degradation of the pretreated plastics using enzymatic cocktails followed by consortium of strains, (ii) separation of the biomass and residual plastic components from the nutrient-rich fluid (using disk centrifugation), and (iii) microbial PHB/rhamnolipid/nanocellulose production using the nutrient-rich effluent stream of the first reactor as influent. The biomass will be dewatered (and dried if required) and send to AIT and AIM for analysis and processing. Feedback of the bioproduct performance characteristics will be used to optimise the production process in conjunction with consortium partners

T7.3. Pilot reactor operation. (Task Leader: AVE. Contributor: IEBT, IMGGE, NTUA, SDU, MLS, TCD). Starter inoculums, consisting of the enzymatic cocktails and strain consortia recommended from WP6, will be supplied to AVE



to start the first reactor (biodegradation of pre-treated plastics) and the second reactor (bioproduction of desired end-product). The inocula will consist of the lab-scale reactor effluents where the constituent microorganisms are already adapted to each other and the process conditions. The inocula will be used to start the pilot-scale reactor using at least 1-10% v/v inoculum, consisting of viable biomass in the range of 2-3 g VSS/L. The influent stream will consist of pre-treated plastics, optimized during previous WPs and produced in sufficient amounts to enable operation of the pilot with predetermined active volume and for a predetermined duration. The fully characterised pre-treated plastics will be supplied by consortium partners. AVE will add micronutrients and nitrogen and phosphorus sources as specified.

T7.4. Monitoring of pilot reactor operation and production process. (Task Leader: AVE. Contributor: IEBT, IMGGE, NTUA, SDU, MLS, AIT )The microbial community composition can be monitored by amplicon sequencing. The degradation of pre-treated plastic will be measured using respirometry methods. Extraction and purification of PHB, nanocellulose, and rhamnolipids will be carried out using environmentally friendly protocols developed in WP6 ready for characterization and processing testing.

T7.5. Extensive chemical, mechanical, thermal and aging characterization and analysis. (Task Leader: AIT. Contributor AVE, IEBT, LOG) Chemical Analysis: A series of chemical analysis including Fourier Transform Infrared Spectroscopy (FTIR), Gel Permeation Chromatography (GPC), Gas Chromatography Mass Spectrometry (GPC-MS) and X-ray diffraction analysis will be carried out to provide information on the bioproduct chemical structure, MW and MW distribution, identification of the chemical composition and to study biopolymer crystallinity all of which are important parameters for processing and establishing end-use applications. A further test important to support this information profile of each of the bioproducts includes: goniometry to measure biopolymer surface hydrophilicity and hydrophobicity, Karl Fischer Colorimetry to test the moisture content of the biopolymers, and High performance liquid chromatography (HPLC) which can establish the purity and identify components in the biopolymers. Morphological Analysis: Morphological studies will be carried out using Scanning Electron Microscopy (SEM) that will enable high resolution surface analysis and provide elemental information on the biopolymer composition. Thermal Characterisation: Thermal analysis will provide information on the thermal stability and degradation, phase transition, and rheology. This will be carried out using Differential scanning calorimetry (DSC), Melt flow index (MFI) to measure the ease of flow of the melt of the biopolymers, and Thermogravimetric analysis (TGA) which will provide information on the thermal stability of the biopolymers. Mechanical Characterisation: Mechanical analysis will include: rheometry for determination of the storage modulus, loss modulus, viscosity and shear strength of the biopolymers, Dynamic Mechanical Thermal Analysis (DMTA) for measurements of the mechanical and thermal properties of the biopolymers. Tensile testing will be used to measure the tensile strength and elongation characteristics of the biopolymers. Compression testing will measure the compression properties for the biopolymers. 3-point bend flexural testing provides measurements of the flexural strength and modulus of the biopolymers. Packaging related tests include weld strength test, tear strength test, bond strength test. Impact resistance will be used for toughness testing. Hardness test including Shore A and Shore D hardness test will be used to further characterise the biopolymers. Accelerated aging analysis will include shelf life testing to determine lifespan of a potential product. Accelerated weathering will be used to establish optical changes and mechanical deterioration of biopolymers for a long period of exposure to UV light, 1000 hours test of Xenon Arc. Accelerated weathering equivalents to 2 years northern hemisphere natural environmental exposure. Weatherometer testing will be used for the photodegradation of the biopolymers.

T7.6. Demonstration of pilot production of PHB and nanocellulose for thin biopolymer film production for applications such as food packaging and rhamnolipids for pharmaceutical products. (Task Leader: LOG. Contributor: AVE IEBT, IMGGE, NTUA, AIT and AIM). PHB and nanocellulose will be processed using hotmelt extrusion/extrusion blow molding and the processing parameters optimised for thin film production. The materials performance of the thin films generated will be compared with the equivalent recalcitrant plastics currently in use in the relevant market applications. Packaging design factors will be formulated in order to optimise the packaging for food contact applications. Extrusion blow molding studies will facilitate comparison with existing commercial materials in order to adjust process parameters and understand material behavior based on the input of WP 6. Prototype mold and parts design and construction will be considered in order to adapt it to the material requirements. Process parameters and suitability of the material rheology will be provided and the information fed back into WP6 to enable biosynthesis improvements. Sample packing will be produced and characterised and customized into final geometries.

T7.7. Life Cycle Analysis of BioICEP technology and products. (Task Leader: TCD. Contributor: AVE IEBT, AIT, LOG and AIM). A life cycle inventory and the life cycle impact assessment will be carried out to identify, quantify, check, and evaluate the environmental impact of the BioICEP technology. This critique and recommendations will be analysed and opportunities to reduce the environmental impact will be carried out. Life cycle analysis will be used to benchmark the BioICEP environmental performance against existing and emerging plastics degradation and recycling technologies.



### Participation per Partner

Partner number and short name	WP8 effort
1 - AIT	8.00
2 - ACTECO	4.00
3 - AIMPLAS	6.00
4 - AVECOM	20.00
6 - IMGGE	10.00
7 - IBET	9.00
12 - TCD	5.00
<b>Total</b>	<b>62.00</b>

### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D8.1	Operation of modular integrated pilot scale plant	4 - AVECOM	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D8.2	Pilot production of high performance PHB and nanocellulose	4 - AVECOM	Report	Confidential, only for members of the consortium (including the Commission Services)	40
D8.3	Report on Life Cycle Analysis study	12 - TCD	Report	Confidential, only for members of the consortium (including the Commission Services)	44

### Description of deliverables

#### Deliverables:

D7.1 Operation of modular integrated BioICEP pilot scale plant demonstrating the biocatalytic and microbial breakdown of 20%+ of mixed plastics. (Month 36)

D7.2. Small-scale pilot production of high performance PHB and nanocellulose for applications such as food packaging and rhamnolipids for pharmaceutical applications. (Month 40)

D7.3 Report on Life Cycle Analysis study demonstrating the low environmental impact of BioICEP and its favourable position compared with current and emergent competitor technologies. (Month 44)

D8.1 : Operation of modular integrated pilot scale plant [36]

Report on the operation of modular integrated BioICEP pilot scale plant demonstrating the biocatalytic and microbial breakdown of 20%+ of mixed plastics.

D8.2 : Pilot production of high performance PHB and nanocellulose [40]

Report on small-scale pilot production of high performance PHB and nanocellulose for applications such as food packaging and rhamnolipids for pharmaceutical applications.

D8.3 : Report on Life Cycle Analysis study [44]

Report on Life Cycle Analysis study demonstrating the low environmental impact of BioICEP and its favourable position compared with current and emergent competitor technologies.

#### Schedule of relevant Milestones

<b>Milestone number<sup>18</sup></b>	<b>Milestone title</b>	<b>Lead beneficiary</b>	<b>Due Date (in months)</b>	<b>Means of verification</b>
MS10	Pilot plant for BioICEP technology	4 - AVECOM	48	Demonstration of the BioICEP technology at pilot level at TRL5 - TRL6

<b>Work package number</b> <sup>9</sup>	WP9	<b>Lead beneficiary</b> <sup>10</sup>	3 - AIMPLAS
<b>Work package title</b>	Dissemination, Exploitation, and Communication		
<b>Start month</b>	1	<b>End month</b>	48

### Objectives

O8.1 Communication & stakeholder engagement  
O8.2 Dissemination  
O8.3 Exploitation  
O8.4 Industry engagement  
O8.5 Business plan development

### Description of work and role of partners

#### **WP9 - Dissemination, Exploitation, and Communication** [Months: 1-48]

**AIMPLAS**, AIT, ACTECO, TUC, IMGGE, IBET, LIT, LOGOPLASTE, NTUA, TCD

AIM leads WP8 and all partners contribute to the tasks. The international partners SDU, CAS and BIT are linked to AIT and AIM for the work in WP 8.

The international partner contributions to the task are as follows:

SDU: Contributing to T8.1-T8.3

CAS: Contributing to T8.1-T8.3

BIT: Contributing to T8.1-T8.3

This WP encompasses the development and delivery of the project Plan for Exploitation of the Results and Dissemination (PERD). WP8 comprises of interlinked actions designed to ensure the successful exploitation of the projects technical innovations. All these activities will lead to strengthen the Europe-China cooperation. A business plan will also be developed demonstrating BioICEP's cost efficient biotransformation of waste plastics into bio products with high performance properties for applications such as food packaging.

Description of work and role of participants: This WP encompasses the development and delivery of the project Plan for Exploitation of the Results and Dissemination (PERD). WP8 comprises of interlinked actions designed to ensure the successful exploitation of the projects technical innovations. All these activities will lead to strengthen the Europe-China cooperation. A business plan will also be developed demonstrating BioICEP's cost efficient biotransformation of waste plastics into bio products with high performance properties for applications such as food packaging.

Objectives:

O8.1 Communication & stakeholder engagement

O8.2 Dissemination

O8.3 Exploitation

O8.4 Industry engagement

O8.5 Business plan development

T8.1 Communication & stakeholder engagement strategy (M1 – M48) Task Leader: AIM. Task Contributors: All partners

As detailed in Section 2.2, the communication strategy will be reflected in a Communication Plan (CP) (CP, D8.2.) developed by AIM through the engagement of all relevant partners to plan the use of resources and communication of the projects results through the channels effectively. In addition an effective Stakeholder Engagement Plan (SEP, D8.2) will be developed. Both combined strategies will ensure effective communication of the project throughout the project geared towards engaging the stakeholders and the relevant different profiles (targeted audiences). The communication strategy will address: communication objectives and approach, target audience (Special Interest Groups and public at large), media channels and tools, messages, activities, materials, and Key Performance indicators (KPIs). The diverse communication activities will have different audience, so the strategy will also establish how resources, communication channels and messages will be used depending on the target audience:

- Plastic producer industries, microorganisms' industries, bioplastic manufacturer Industry in Europe and China and technology providers;
- Technical experts, researchers and scientific community in Europe and China;
- Policy makers, authorities and public bodies in Europe and China; and
- General public. In particular young audience will be targeted as the future global citizens.

1. Four online training webinars will be launched and promote in schools and high schools to disseminate the project outcomes among the young citizens.
2. The results of the project will also be disseminated to the wide public through four entertaining short stories and documentaries making European citizens to understand the project innovation, its achievements and the lessons learnt during it, trying to raise general public's awareness in plastic waste management issues and to motivate early adopters.
3. Use of online communication tools: YouTube, Facebook, Twitter and LinkedIn will be used to communicate the project. Updates will be done on a monthly basis.
4. Project website: The BioICEP website will serve as the main interaction and collaboration platform to introduce the project and its progress, the Consortium, BioICEP innovative technologies. The website will allow the public to consult non-confidential information about the project (description, benefits, technologies etc.). The project website will be available in English and Chinese. The partners will publish a description of their role, the project description, and a link to their respective websites. The website will be continuously updated throughout the project as new information becomes available. It will remain active for at least five years after the project's completion by continued maintenance from AIM. Within the website, a section will be devoted to network with other H2020 projects (links to these projects, short description, advertising of liaison activities etc.)
5. Quantified outputs: more than 8,000 visits per year of the project website
6. Notice Boards: Notice boards will be displaced at each demonstration site. Each notice board will provide basic information of the project including the beneficiaries and will explain the importance and benefits of the BioICEP technology.
7. In parallel to these communication activities, according to the H2020 guidelines, a Data Management Plan (DMP, D8.3) will be created by AIM by using the FAIR EC system. A deliverable (first version) will be provided in month 6 and updated subsequently.

#### T 8.2 Dissemination activities (M1 – M48) Task Leader: AIM. Task Contributors: All partners

A series of dissemination activities will be implemented in order to diffuse the objectives and outcomes of BioICEP to the public, industry, and the scientific community. This includes the following activities to develop BioICEP identity:

1. Publications in scientific journals, presentations in scientific conferences, and at industry related events: The BioICEP scientific results will be published at international peer reviewed journals with significant impact factor, open access will also be considered without affecting IPRs. Furthermore, presentations of the BioICEP results will take place at international conferences and at industrial fairs and exhibitions (a specific plan including target events has been included in Section 2.2).
2. Plenary BioICEP Conference, with broad target audience of > 50 participants: A final conference will be organized by AIT, in which the outcomes of BioICEP will be made visible to the key target groups and industrial stakeholders showcasing the results. The conference will provide a summary of technical results and the case scenarios for the implementation of BioICEP. There will be also interactive sections allowing target users to receive feedback on suitability of BioICEP technology for their specific needs.
3. Workshop: Two workshops will be organised as stand-alone events by AIT and AIM to ensure optimal visibility and maximise the impact of the project on the community.
4. The Chinese partners will also host a workshop to disseminate the project results and strengthen scientific cooperation between Europe and China. The following audiences will be targeted: industries, universities and scientific partners, local authorities, and policy makers. During the event a matchmaking event will be organised to maximize the cooperation in future project and direct business.

Quantified outputs: more than > 60 scientific papers, > 30 presentations in scientific conferences and at industry related events, > 50 participants in the final conference.

#### T 8.3 Exploitation, innovation and IPR management (M1 – M48) Task Leader: AIM. Task contributors: All partners

This task consists of a value chain analysis to identify key opportunities and barriers for BioICEP, including most likely scenarios for future value chains. The BioICEP business model will be defined to capture opportunities and overcome market entry barriers, thus maximizing its impact across value chains. A Dissemination and Exploitation Plan (PDER, D8.4 - D8.6) will be produced capturing all relevant segments of the value chain, their engagement, and target activities to actualize the strategy.

All patentable results will be patented by the owner or joint-owner(s). An Intellectual Property Rights and Exploitation Board (IPREB) will be chaired by the project coordinator and operated by AIM. It will be made-up of one key partner for each of the Exploitable Results, representing all the scientific and industrial partners' business interests. The IPREB will boost the proper innovation and IPR management during the project by completing the management of the commercial and industrial exploitation of the key results. The IPREB will provide a robust internal consultancy to the Executive Board in order to keep priority on the IPR issues. Exploitation plan will include value proposition (D8.4, D8.5 & D8.6) due in months 12, 36 and 48. The exploitation plans will describe the route for exploitation that will depend on the type

of results. To that aim, an Innovation Management Board (IMB), led by AIT has been added to the project management structure providing technical, legal and economic expertise in technology transfer and supporting guidance on IPR and Innovation Management, following the Guideline for IPR rules in H2020. Thus, the IMB is made up of the experts (leaders) of those fields. This group will guarantee that the Plan for the Dissemination and Exploitation of Results (PDER) are coherent and well interconnected.

This task also includes the activities for developing business models capable of assessing economic impact and to estimate economic indicators in relevant scenarios for implementation. The outcome will be model business plans for the relevant scenarios. The innovation margins of each Key Exploitable Result will be identified and translated to market terms. SWOT and Business Model Canvas (BMC) will ensure the prerequisite actions to be taken in order to reinforce the Exploitation Strategy with qualitative and quantitative measures for each partner.

In addition, a Technology watch service will be carried out based on AIM proprietary software SoftVT <http://www.observatorioplastico.com/> that will provide updated information of the state of the technology, patents, market, etc, related to BioICEP innovations.

To maximise the technology transfer of innovative processes developed by BioICEP the PM, PC and DE manager will organise a BioICEP Internship Programme available to researchers within and beyond the BioICEP consortium. It is envisaged that 10 internships will facilitate the transfer of skills and know how between consortium members and the greater research community. The primary objective of the BioICEP Internship Programme will be to support early stage researchers in the sector. Second, this programme will further enhance the dissemination and exploitation activities of the expertise contained within the established BioICEP consortium thus promoting uptake of results generated. Third, the internship programme will impart the knowledge required to produce independent researchers who will facilitate the growth and expansion of BioICEP beyond the lifetime of the Horizon 2020 programme.

T 8.4 Industry Engagement Strategy Task Leader: AIT. Task Contributors: All partners. Local and major global recycling and plastic manufacturing companies will be contacted and informed on an ongoing basis of the BioICEP technology developments. Meetings will secure interest and commercial support while providing important direction to steer the technology developments inline with consumer and industry demands. These engagements will also provide companies with increased confidence to adopt new bioproducts, from a wide range of different producers. Our industry engagements will be facilitated by the wide commercial client base of consortium partners, including AIT, AIM, IEBT and SME partners.

T8.5. Business model development. (Task Leader: AIT. Contributor: AVE IEBT, MLS LOG, ACT and AIM). Development of BioICEP to high TRL upon completion of this project at TRL 5-6 will be derived directly from our business model. Revenues will be received from two sources: i) from the sale of high performance biopolymers and bioproducts, and ii) from industry needing to dispose mixed plastic waste. This will reduce the costs of commercialisation and allows BioICEP to generate revenues throughout the commercialisation process. The identification of high potential revenue stream and potential prospective licensees for different elements of the technology will feed back into the business development activities.

#### Participation per Partner

Partner number and short name	WP9 effort
1 - AIT	13.00
2 - ACTECO	2.00
3 - AIMPLAS	24.00
5 - TUC	2.00
6 - IMGGE	2.00
7 - IBET	2.00
8 - LIT	4.00
9 - LOGOPLASTE	2.00
11 - NTUA	2.00

Partner number and short name	WP9 effort
12 - TCD	2.00
<b>Total</b>	<b>55.00</b>

### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D9.1	Project website	3 - AIMPLAS	Websites, patents filling, etc.	Public	2
D9.2	Communication Plan (CP)	3 - AIMPLAS	Websites, patents filling, etc.	Confidential, only for members of the consortium (including the Commission Services)	4
D9.3	Data Management Plan (DMP)	3 - AIMPLAS	Report	Confidential, only for members of the consortium (including the Commission Services)	6
D9.4	Preliminary Plan for Dissemination and Exploitation of Results progress (PDER)	3 - AIMPLAS	Report	Confidential, only for members of the consortium (including the Commission Services)	12
D9.5	PDER	3 - AIMPLAS	Report	Confidential, only for members of the consortium (including the Commission Services)	36
D9.6	Open Research Data Pilot	1 - AIT	ORDP: Open Research Data Pilot	Public	6
D9.7	Technology Watch Service report	1 - AIT	Report	Confidential, only for members of the consortium (including the Commission Services)	48
D9.8	Business Model presenting the go to market potential	1 - AIT	Report	Confidential, only for members of the consortium (including the Commission Services)	48

### Description of deliverables

Deliverables:

D8.1 Project website (updated throughout the project). (Month 3)

D8.2 Communication Plan (CP) & Stakeholder Engagement Plan (SEP). (Month 4)

D8.3 Data Management Plan (DMP). (Month 6)  
D8.4. Preliminary Plan for Dissemination and Exploitation of Results progress (PDER). (Month 12)  
D8.5. Mid-Term PDER progress. ( Month 36)  
D8.6. PDER final version. (Month 48)  
D8.7 Technology Watch Service report. (Month 46)  
D8.8 Report on industry engagement strategy. (Month 48)  
D8.9. Business plan presenting the go to market potential and market projections for BioICEP. (Month 48).

D9.1 : Project website [2]

The BioICEP project website will allow the public to consult non-confidential information about the project (description, benefits, technologies etc.). The project website will be available in English and Chinese.

D9.2 : Communication Plan (CP) [4]

Communication Plan (CP) will be developed involving the engagement of all relevant partners to plan the use of resources and communication of the projects results through a range of channels effectively

D9.3 : Data Management Plan (DMP) [6]

BioICEP will develop and implement Data Management Plans (DMPs) using the FAIR EC system

D9.4 : Preliminary Plan for Dissemination and Exploitation of Results progress (PDER) [12]

A Dissemination and Exploitation Plan (PDER) will be produced capturing all relevant segments of the value chain, their engagement, and target activities to actualize the strategy

D9.5 : PDER [36]

A mid term and final report updating the rolling Plan for the Dissemination and Exploitation of Results (PDER) will be provided at month 36 and 48 respectively

D9.6 : Open Research Data Pilot [6]

AIT will publish will make available all peer-reviewed scientific publications relating to its results where feasible free of charge by online access for any user through the AIT repository Research@Thea: AIT

D9.7 : Technology Watch Service report [48]

A technology Watch Service report will be provided detailing the industry engagement strategy and monitoring industrial interaction with the BioICEP project

D9.8 : Business Model presenting the go to market potential [48]

The BioICEP business model will be defined to capture opportunities and overcome market entry barriers, thus maximizing its impact across value chains.

#### Schedule of relevant Milestones

Milestone number <sup>18</sup>	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
MS11	Project Website	3 - AIMPLAS	3	Online operation of project website
MS12	First partner approved PDER	3 - AIMPLAS	18	First PDER approved by all partners



<b>Work package number</b> <sup>9</sup>	WP10	<b>Lead beneficiary</b> <sup>10</sup>	1 - AIT
<b>Work package title</b>	Ethics requirements		
<b>Start month</b>	1	<b>End month</b>	48

### Objectives

- Ensure compliance with the 'ethics requirements' set out in this work package.
- Appointment of an ethics advisor to ensure the ethical compliance of the BioICEP project
- Documentation and file maintenance of required risk analysis, ethics approvals and authorisations, licencing and legal obligation compliance

### Description of work and role of partners

#### **WP10 - Ethics requirements** [Months: 1-48]

##### **AIT**

This work package sets out the 'ethics requirements' that the project must comply with.

The 'ethics requirements' that the BioICEP project must comply with are included as deliverables in this work package.

- An ethics advisor will be appointed from the AIT ethics committee. This advisor will maintain an overview of the work throughout the whole course of the BioICEP project and will assist in checking for compliance with ethical standards relevant, facilitating the probity of the BioICEP research activities and reporting to the co-ordinator and to the Commission/Agency.

- For Serbia and China as non-EU countries within the BioICEP consortium a risk-benefit analysis will be provided on their research activities, which involve micro-organism sample collection for plastic waste sites and investigation and promotion of these microbes for waste plastic biodegradation and fermentation.

- o Copies of ethics approvals and other authorisations or notifications as required will be provided and maintained on file.
- o A documented opinion from the AIT Ethics committee or other appropriate ethics structure in an EU consortium country confirming that the research activity could have been legally carried out in an EU country will be provided and kept on file.

- o documentation demonstrating compliance with the UN Convention on Biological Diversity will be provided

- Import of micro-organism materials from Serbia or China into the EU and or export micro-organism materials from the EU to Serbia or China will be fully licenced as required and copies of any import and export licences will be kept on file.

- Further information about the possible harm to the environment caused by the research and the measures that will be taken to mitigate the risks will be kept on file and submitted as a deliverable.

In addition to the BioICEP research activity being accepted and complying with the legal obligations the non-EU countries the activities are also allowed in at least one EU Member State of the BioICEP consortium. The consortium partners will confirm this condition is met as part of the grant agreement

In the case that researchers travel to work in Serbia or China a risk assessment will be undertaken taking appropriate safety measures into account.

### Participation per Partner

<b>Partner number and short name</b>	<b>WP10 effort</b>
1 - AIT	1.00
<b>Total</b>	1.00



### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D10.1	NEC Requirement 1	1 - AIT	Other	Confidential, only for members of the consortium (including the Commission Services)	3
D10.2	EPQ- Requirement No. 2	1 - AIT	Report	Confidential, only for members of the consortium (including the Commission Services)	48

### Description of deliverables

1. In case activities undertaken in Serbia and China raise ethics issues, the BioICEP consortium partners will ensure that the research conducted outside the EU is legal in at least one EU Member State.
2. Details on the materials which will be imported to/exported from the EU must be kept on file
3. Copies of import/export authorisations, as required by national/EU legislation will be kept on file.
4. Further information about the possible harm to the environment caused by the research and the measures that will be taken to mitigate the risks will be kept on file.

#### D10.1 : NEC Requirement 1 [3]

BioICEP consortium partners will ensure that the research conducted outside the EU is legal in at least one EU Member State and files will be kept on the authorisations and details of materials which will be imported to/exported from the EU. 1. In case activities undertaken in Serbia and China raise ethics issues, the BioICEP consortium partners will ensure that the research conducted outside the EU is legal in at least one EU Member State. 2. Details on the materials which will be imported to/exported from the EU must be kept on file 3. Copies of import/export authorisations, as required by national/EU legislation will be kept on file.

#### D10.2 : EPQ- Requirement No. 2 [48]

A file will be kept on further information and risk analysis on the possible harm to the environment caused by the research and the measures that will be taken to mitigate any risks.

### Schedule of relevant Milestones

Milestone number <sup>18</sup>	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification
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### 1.3.4. WT4 List of milestones

Milestone number <sup>18</sup>	Milestone title	WP number <sup>9</sup>	Lead beneficiary	Due Date (in months) <sup>17</sup>	Means of verification
MS1	Completion of all reporting	WP2	1 - AIT	48	All project plans delivered and reporting complete
MS2	Plastic waste feedstock	WP3	12 - TCD	12	Categorised, characterised and prepared post use plastic waste feedstock supply to partners
MS3	Optimised Pretreatment process	WP3	12 - TCD	24	Optimised and integrated pretreatment process for mixed plastics
MS4	Model substrates for plastic degradation assays	WP4	6 - IMGGE	6	Model plastic substrates for assaying enzyme & microbial activities
MS5	Novel degrading enzyme cocktails	WP4	6 - IMGGE	24	Novel enzymatic cocktails with high degradation activities
MS6	Platform for plastic waste bioconversion	WP4	6 - IMGGE	46	Microbial platform for simultaneous degradation & synthesis
MS7	Catalogue of best microbial degraders	WP5	8 - LIT	36	Catalogue of Identified best microbial biodegraders and value-added biomolecule producers
MS8	Optimal performing Microbial Consortia	WP6	10 - MicroLife	46	Optimal performing Microbial Consortia degrading greater than 20% of mixed plastics
MS9	Established bioproduct bioprocess	WP7	7 - IBET	48	Established bioprocess for the production of high performance PHB, nanocellulose and rhamnolipid bioproducts for high need market segments within the food and pharmaceutical industry
MS10	Pilot plant for BioICEP technology	WP8	4 - AVECOM	48	Demonstration of the BioICEP technology at pilot level at TRL5 - TRL6
MS11	Project Website	WP9	3 - AIMPLAS	3	Online operation of project website
MS12	First partner approved PDER	WP9	3 - AIMPLAS	18	First PDER approved by all partners
MS13	BioICEP Conference		3 - AIMPLAS	40	Launch of BioICEP Conference

### 1.3.5. WT5 Critical Implementation risks and mitigation actions

Risk number	Description of risk	WP Number	Proposed risk-mitigation measures
1	Delays of key deliverables	WP2	In the event the deadline cannot be met, a provisional draft will be developed allowing any interdependent actions to be carried out. Milestones are placed to proactively control part of the work program where inter-dependencies may become critical and dedicated risk management strategies are foreseen for specific critical milestones.
2	IPR or other conflict amongst the partners	WP2	The frequent communication foreseen in task 1.2 will allow early detection of potential issues. The coordinator will intervene to facilitate dialogue between involved partners, at the highest level. As a last resort, the conflict resolution measures clearly assigned and agreed upon in the consortium agreement will be activated.
3	Management issues due to Cross continental consortium	WP2	AIT has a dedicated EU-China engagement officer who has been instrumental in developing the strong relationship between the Chinese partners within the consortium. Hence this relationship will be further fostered throughout the project. The proposed working groups that will be chaired by the Technical Manager, have relevant representatives of the most relevant disciplines in order to assure the required leadership.
4	Financial risk.	WP1	Administrative/financial management will maintain a close financial monitoring process so as to constantly assess financial progress and be able to identify early signs of concern.

### 1.3.6. WT6 Summary of project effort in person-months

	WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8	WP9	WP10	Total Person/Months per Participant
1 - AIT	✓	39	24	0	6	6	8	8	13	1	105
2 - ACTECO		0.50	13	0	0	0	0	4	2	0	19.50
3 - AIMPLAS		3	38	0	0	0	14	6	24	0	85
4 - AVECOM		2	0	3	0	12	4	20	0	0	41
5 - TUC		2	9	0	12	0	0	0	2	0	25
6 - IMGGE		2	0	90	6	18	56	10	2	0	184
7 - IBET		4	1	3	0	6	12	9	2	0	37
8 - LIT		2	0	0	56	10	0	0	4	0	72
9 - LOGOPLASTE		1	4	0	0	0	23	0	2	0	30
10 - MicroLife		2	0	8	8	18	2	0	0	0	38
11 - NTUA		2	0	19	8	7	0	0	2	0	38
12 - TCD		2	38	0	0	0	7	5	2	0	54
<b>Total Person/Months</b>		61.50	127	123	96	77	126	62	55	1	728.50

### *1.3.7. WT7 Tentative schedule of project reviews*

No project reviews indicated

### 1. Project number

The project number has been assigned by the Commission as the unique identifier for your project. It cannot be changed. The project number **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

### 2. Project acronym

Use the project acronym as given in the submitted proposal. It can generally not be changed. The same acronym **should appear on each page of the grant agreement preparation documents (part A and part B)** to prevent errors during its handling.

### 3. Project title

Use the title (preferably no longer than 200 characters) as indicated in the submitted proposal. Minor corrections are possible if agreed during the preparation of the grant agreement.

### 4. Starting date

Unless a specific (fixed) starting date is duly justified and agreed upon during the preparation of the Grant Agreement, the project will start on the first day of the month following the entry into force of the Grant Agreement (NB : entry into force = signature by the Commission). Please note that if a fixed starting date is used, you will be required to provide a written justification.

### 5. Duration

Insert the duration of the project in full months.

### 6. Call (part) identifier

The Call (part) identifier is the reference number given in the call or part of the call you were addressing, as indicated in the publication of the call in the Official Journal of the European Union. You have to use the identifier given by the Commission in the letter inviting to prepare the grant agreement.

### 7. Abstract

### 8. Project Entry Month

The month at which the participant joined the consortium, month 1 marking the start date of the project, and all other start dates being relative to this start date.

### 9. Work Package number

Work package number: WP1, WP2, WP3, ..., WPn

### 10. Lead beneficiary

This must be one of the beneficiaries in the grant (not a third party) - Number of the beneficiary leading the work in this work package

### 11. Person-months per work package

The total number of person-months allocated to each work package.

### 12. Start month

Relative start date for the work in the specific work packages, month 1 marking the start date of the project, and all other start dates being relative to this start date.

### 13. End month

Relative end date, month 1 marking the start date of the project, and all end dates being relative to this start date.

### 14. Deliverable number

Deliverable numbers: D1 - Dn

### 15. Type

Please indicate the type of the deliverable using one of the following codes:

R	Document, report
DEM	Demonstrator, pilot, prototype
DEC	Websites, patent filings, videos, etc.
OTHER	
ETHICS	Ethics requirement
ORDP	Open Research Data Pilot
DATA	data sets, microdata, etc.

#### 16. Dissemination level

Please indicate the dissemination level using one of the following codes:

- PU        Public
- CO        Confidential, only for members of the consortium (including the Commission Services)
- EU-RES   Classified Information: RESTREINT UE (Commission Decision 2005/444/EC)
- EU-CON   Classified Information: CONFIDENTIEL UE (Commission Decision 2005/444/EC)
- EU-SEC   Classified Information: SECRET UE (Commission Decision 2005/444/EC)

#### 17. Delivery date for Deliverable

Month in which the deliverables will be available, month 1 marking the start date of the project, and all delivery dates being relative to this start date.

#### 18. Milestone number

Milestone number: MS1, MS2, ..., MSn

#### 19. Review number

Review number: RV1, RV2, ..., RVn

#### 20. Installation Number

Number progressively the installations of a same infrastructure. An installation is a part of an infrastructure that could be used independently from the rest.

#### 21. Installation country

Code of the country where the installation is located or IO if the access provider (the beneficiary or linked third party) is an international organization, an ERIC or a similar legal entity.

#### 22. Type of access

- VA        if virtual access,
- TA-uc    if trans-national access with access costs declared on the basis of unit cost,
- TA-ac    if trans-national access with access costs declared as actual costs, and
- TA-cb    if trans-national access with access costs declared as a combination of actual costs and costs on the basis of unit cost.

#### 23. Access costs

Cost of the access provided under the project. For virtual access fill only the second column. For trans-national access fill one of the two columns or both according to the way access costs are declared. Trans-national access costs on the basis of unit cost will result from the unit cost by the quantity of access to be provided.

# History of Changes

Notes: - Changes relate to comments from the European Commission and are indexed according to point numbers relating to each comment

- Outside the changes listed in the “history of Changes” the “track Changes” and minor typo corrections, no other changes have been made to this document.

a)	Preparation of Part B of Annex 1. (P.13) Tables 3.1a, 3.1b, and 3.1c removed from section 3.1. Tables 3.2a and 3.2b removed from section 3.2. Table 3.4a removed from section 3.4	
b)	Confirmation IPR will take Grant Agreement Articles 23a-31 into account and be respected (P9.)	37
c)	Clarification only communications activities within the project’s lifetime will be covered (P20.)	48
d)	Updated Organigram of BioICEP diagram to clarify EU-China Engagement (P21.)	53
e)	Clarification of international partners participation in the project bodies (P21.)	54-55
f)	Amended Table 37: 3.4b (other direct costs) (P3., P7. and P14.)	61
g)	Addition of Section 4. Members of the Consortium including 4.1 Participants and 4 .2. Third parties involved the project (including use of third party resources)	63
h)	Clarified references to EU entities only or to EU beneficiaries + international partners throughout the document including defining terms at the beginning of the document and in table 38 (P19.)	All
i)	Contributions by international partners and linking to coordinator and EU Partners (P4.)	138
j)	Clarification of beneficiary use of in-kind third-party contributions (P. 6)	138
k)	Elaboration with added details, on the objective of developing microorganism communities and regarding the implementation of the work plan (P. 25)	50
l)	How the main results from the project will be used or verified in pilot scale and the data for LCA will match the objective of the proposal. (P.26)	51
m)	How the project will deliver the four specific output indicators listed in the impact section (P. 27)	28
n)	Figures by market segment for assessment of the credibility of the financial projections stated in the business model. (P.28)	41
o)	Elaboration on mitigation measures applied to the identified risks were identified (p.29)	56
p)	Added Section 5 Ethics and security	140
q)	Moved internships opportunities to section 3.3	34,35,38,60



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## LIST OF ABBREVIATIONS AND DEFINITIONS

BioICEP	Bio Innovation of a Circular Economy for Plastics		
NSFC	National Natural Science Foundation of China	EC	European Commission
PE	Polyethylene	GHG	Greenhouse Gas
PP	Polypropylene	CAGR	Compound Annual Growth Rate
PVC	Polyvinyl Chloride	iCHIP	Isolation Chip
PS	Polystyrene	LDPE	Low Density Polyethylene
PET	Polyethylene Terephthalate	LLDPE	Linear Low Density Polyethylene
PU	Polyurethane	HDPE	High Density Polyethylene
PLA	Polylactic acid	PHA	Polyhydroxyalkanoate
PBS	Polybutylene succinate	PCL	Polycaprolactone
RPO	Research Performing Organisation	PHB	Polyhydroxybutyrate
RTO	Research and Technology Organisation	SME	Small- and Medium-sized Enterprise
MW	Molecular Weight	UN	United Nations
IP	Intellectual Property	SDGs	Sustainable Development Goals
Beneficiaries	EU entities	International Partners	Chinese Entities
All Participants	EU and Chinese Entities	Consortium	All Participants

## Introduction to the technological concept

The Bio Innovation of a Circular Economy for Plastics (BioICEP) consortium is a pan European-Chinese collaboration formed to reduce the burden of plastic waste in the environment. The countries have been selected to represent different mixed plastic pollution environments, with specific partners chosen which have the expertise and facilities to carry out the necessary technical innovations. A number of innovative booster technologies are at the core of this solution accentuating, expediting, and augmenting mixed plastics degradation to levels far in excess of those current achievable. Our approach is a triple-action depolymerisation system where mixed plastic waste will be broken down in three consecutive processes: 1) mechano-biochemical disintegration processes, including a new proprietary sonic-green-chemical technology to reduce the molecular weight (MW) of the base polymer making it amenable to biodegradation; 2) biocatalytic digestion, with enzymes enhanced through a range of innovative techniques including accelerated screening utilising novel fluorescent sensors coupled with directed evolution; and 3) microbial consortia developed from best in class single microbial strains, which when combined lead to highly efficient degradation of mixed plastic waste streams. The outputs from this degradation process will be used as building blocks for new polymers or other bioproducts to enable a new plastic waste-based circular economy. The BioICEP technology has the potential to lead to dramatic financial savings on the overall social and environmental pollution plastic pollution costs, estimated to be \$139bn a year by Trucost, a research arm of Standard & Poors.

**Acceleration of environmental waste plastic degradation** The innovative accelerated and directed evolution of both enzymatic and microbial consortia combined with novel pre-treatments are designed to overcome the current century-long degradation challenges of petroleum-based plastics. Novel *in situ* biosensors will accelerate the identification of high-performing microbial strains and enzymatic activities. These strains will be collected from the most plastic polluted global sites where microbes have had the longest and most intensive opportunities to evolve. Global blackspots for environmental pollution include *"the Chinese Yangtze River which has an annual input of 330 thousand tonnes of plastic discharged into the East China Sea and the Danube river in eastern Europe where 530–1,500 tonnes of plastic discharged into the Black Sea annually."* Consortium partners in China and Serbia will facilitate access and collection at these sites to dramatically increase the discovery rate of new higher-efficiency plastics degrading strains eclipsing the current exhaustive search process. Fast-tracked and directed evolution will be applied including novel CRISPR-9Cas technology to surmount the intrinsically slow natural evolution progression. Innovative bioengineering strategies will be employed to ensure the effective collective functioning of microbial and fungal strains for the degradation of mixed plastic environmental waste. The combination of mechano-biochemical disintegration, biocatalytic digestion, and novel microbial consortia will deliver a next generation sustainable and seamless solution for the complete breakdown of at least 20% of mixed waste plastic polymers, ready for reassembly into new bioproducts and equivalent biopolymer plastics. Operation of the BioICEP technology at high pollution sites has the potential to deliver cost savings within local regions of the order of €20 per tonne, which would amount to a €4.2bn annual reduction in the socio-economic impacts along the Yangtze River and the generation of a further €66 M in high value replacement bioplastics and new bioproducts.

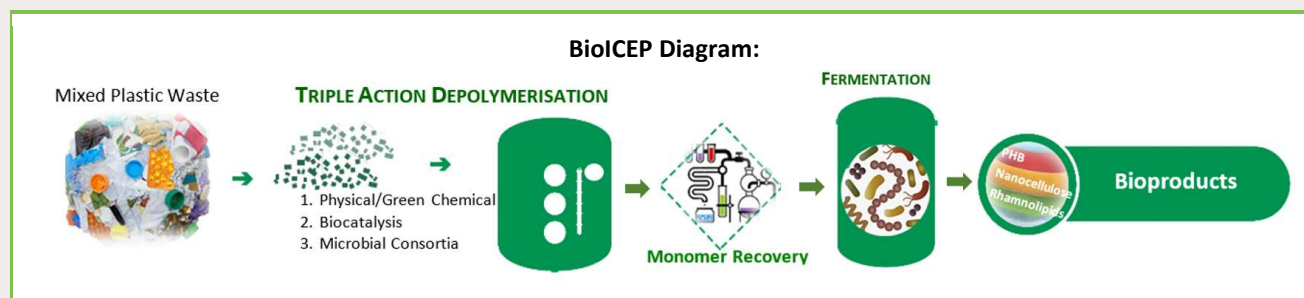


Figure 1 - Technological Concept & Operating Model

**Regeneration into high market demand biodegradable products** Systemic biology, microbial consortia and enzyme cocktails that are enriched and evolved for increased yields of high-quality bioproduct production from the degraded waste plastic carbonaceous constituents will be applied to produce in-demand products. These products will include Polyhydroxybutyrate (PHB) and nanocellulose for compostable food packaging

applications, compatibilised bioplastic blends for the production of 3D printing filaments, and rhamnolipid biosurfactants for the cosmetic and pharmaceutical industries. This step will complete the life cycle of plastics waste in tandem with nature's bio- generation, degradation, and regeneration cycle.

**Cleanup of Global Waste Plastic Burden** BioICEP provides a novel solution to mitigate against the unintended consequences of plastic pollution. This highly responsible and sustainable route will redirect our current mixed plastic waste stockpiles, funnelling the resource-rich carbon outputs for the production of naturally biodegradable replacement plastics and bioproducts. This capacity will be fully demonstrated in the planned prototype plant operating at TRL 5/6, which will integrate the production of depolymerised waste mixed plastic feedstock and the subsequent biosynthesis of high-value bioproducts. This design can achieve rapid social and market acceptance as these materials can be used as a direct replacement for petroleum based polymers.

Table 1 – Project at a Glance

## 1.1. Objectives

The project's overall objective is to demonstrate a seamless sustainable route to a circular economy for plastics by developing an advanced energy, carbon, and cost-efficient waste plastic biotransformation into high market demand bioproducts and bioplastics. The consortium brings together leading experts from industry and academia contributing a set of purpose-designed and ground-breaking technologies in order to achieve the following specific objectives:

### STRATEGIC GOAL 1:

**Development of accelerated high-efficiency biodegradation incorporating microorganism communities expressing at least three novel and improved enzymatic activities enabling the degradation of mixtures of plastics.**

Three of our eight WPs (WP 3, WP 4, and WP 5) are dedicated to delivering this highly ambitious and world-leading solution. A strategy of intensified enzymatic digestion followed by elevated microbial degradation will be achieved by implementing the following objectives:

- 1.1. Development of **enzyme cocktails using accelerated screening and performance enhancing bioengineering** for the degradation of pre-treated mixed plastics (Starch- 100%, Polyhydroxyalkanoate (PHA)- 70%, Polyethylene Terephthalate (PET) -50%, Polyurethane (PU)- 30%, Polystyrene (PS) -20%, Polyethylene PE-10%). Means of verification: The degradation of each polymer, as well as the mixture components will be determined by monomer yield, polymer weight loss and CO<sub>2</sub> release measurements. At least three novel and improved enzymatic activities for mixed plastics degradation will be reported (T3.2, T3.3, T3.4, T3.6, D3.3, D3.4, and D3.8. Main Partners: IMGGE, CAS and NTUA)
- 1.2. Develop stabilised biocatalysis technologies to **increase enzymatic activity by 10%; reusability by reducing denaturing rates to <20% resulting in a cost saving of 20%**. Means of verification: Identification of different enzyme sets which are stable under plastic processing conditions of 150-200°C (T3.5 and D3.7. Main Partners: IMGGE and NTUA)
- 1.3. Isolation of new microbes and biobank enrichment using iCHIP technology and identification of optimum strains. Means of verification: Identification of a minimum of ten new plastic degrading microbial strains collected from targeted global polluted sites. (T4.1, T4.2, T4.4, T4.5, D4.4, and D4.5. Main Partners: LIT, MLS, SDU and BIT)
- 1.4. **Designer strain development delivering enhanced plastic hydrolysis using directed evolution and novel CRISPR-Cas genome editing technology.** Means of verification: Metabolic engineering to incorporate three pathways into a single host strain platform for degradation of multiple targeted plastic wastes. (T4.3 and D4.3. Main Partners: SDU, IMGGE, NTUA and BIT)
- 1.5. **Creation of stable mixed microbial communities with degradation capacity which combined with pretreatments will result in at least 20% degradation of mixed plastics.** Means of verification: Formulation techniques and synthetic biology will be applied for the development of mixed microbial inoculants in order to engineer microbial communities for efficient mixed plastic degradation (T5.1-T5.5, D5.2 and D5.3. Main Partners: MLS, LIT, and BIT)

### STRATEGIC GOAL 2:

**Sustainable degradation of at least 20% of mixed plastics.**

This target will be achieved using our triple action sequential depolymerisation approach of 1) powerful mechano-biochemical degradation; 2) intensified biocatalytic digestion; and 3) amplified microbial consortia decomposition.

- 2.1 **Preparation of industry informed mixed waste plastic stocks will be prepared from** Polyethylene (LDPE, LLDPE, HDPE), (PS), (PET), (PU), Polylactic acid (PLA), PHA, HB, starch, Polybutylene Succinate (PBS), and Polycaprolactone (PCL). Means of verification: Preparation of stocks according to commercial recycling industry information. (T2.1 and D2.1. Main Partners: TCD and ACT)
- 2.2 **Development of a novel combination of mechano-biochemical processes for the reduction of mixed plastic polymer MW by 25-50 %** Means of verification: Methods such as ultrasonication and supercritical carbon dioxide will be combined along with UV-assisted photo degradation, microwave thermal degradation. (T2.2, D2.2. Main partners: TCD, AIT, AIM, CUT and ACT)
- 2.3 **Blending of plastics waste with biodegradable/natural polymers, pro-oxidants and unsaturated polymers will be formulated to increase the propensity for degradation by 50%-100%.** Means of verification: Thermally stabilised/cross-linked enzymes blended with the plastics will further propel degradation. (T2.2, T2.4, T4.5 D2.3 and D6.3 Main Partners TCD, IMGGE AIT, AIM)
- 2.4 **Qualitative analysis of plastic breakdown** using a series of physical and chemical analytical techniques. Additionally, biocatalytic and microbial degradation will be analysed at each stage to establish reduction in weight and weight loss efficiency. Means of verification: Time dependent plastic degradation will be determined at each stage using chromatography techniques. Processes will be optimized to achieve at least 20 % weight reduction. (T2.4, T3.5, T4.5, T5.5, T5.7, T7.2, D3.5, D5.4, and D7.1. Main Partners: TCD, LIT, IMGGE, MLS)
- 2.5 **Operation of modular integrated BioICEP pilot scale plant** using pre-treated mixed plastic feedstock. Means of verification: Demonstration of biocatalytic and microbial breakdown of a minimum of 20% of mechano-biochemical pretreated mixed plastics. (T7.1, T7.2, and D7.1. Main Partners, AVE, iBET, IMGGE, AIT). A business plan presented in section 2.2.A.2 outlines the potential of developing the technology to high TRL post project and launching the BioICEP technology as a commercial operation.

### STRATEGIC GOAL 3:

#### **Bioprocessed high value bioproducts including equivalent bioplastics valorising mixed plastic waste.**

Highly sought-after bioproducts are targeted including PHBs and nanocellulose for equivalent plastics with applications in segments such as the food packaging industry and rhamnolipids as important bio-surfactants in the cosmetic and pharmaceutical industry. In addition, polymer compatibilisers will be developed to prepare polymer blends suitable for 3D printing.

- 3.1 **Bioprocessing of four high market demand bioproducts, PHB, nanocellulose and rhamnolipids from degraded plastic waste feedstock, with conversion rates of at least 60%, and compatibilised 3D printing filaments.** Means of verification: Target yields are PHB 70% dry cell weight. Nanocellulose 20 g per L, Rhamnolipids conversion rate of in excess of 60% e.g. 600 g from 1 kg of substrate. Reuse of 80% of non-degraded polymers through compatibilisation and fabrication into filaments for 3D printing or as a contingency beams for the construction industry. (T2.3, T6.1, T6.2, T4.5, T5.5, T5.7, D3.5, D3.6, and D5.4. Main Partners: iBET, LIT, IMGGE, and MLS)
- 3.2 **Development of protocol for optimized downstream processing including harvesting and purification process for biobased products.** Means of verification: Identify operational conditions, monomer concentration and quality, microbial medium composition and polymer feed composition strategies used in the degradation of mixed plastic feedstock. (T6.5, D6.4, iBET, LIT, IMGGE, and MLS)

### STRATEGIC GOAL 4:

#### **Sustainable prototype system plan, paving the way to bring the developed solution to the market, fulfilling current needs, future expectations, and delivering a seamless bio-innovative route for a circular economy for plastics.**

An integrated prototype laboratory set up including: a modular biocatalytic and microbial pretreated plastics degradation bioreactor, biomass separation, and bioproduct fermentation operating at TRL5/6.

- 4.1 **Prototype scale validation of the biodegradation potential of the best-performing biocatalysts and microbial consortia for the breakdown of >20% pretreated mixed plastics into constituents suitable for bioprocessing of high value added products.** Means of verification: Three strains/strain consortia, each producing one of the envisaged microbial products, will be cultivated in 100 L bioreactors to validate the cultivation conditions and define the cultivation protocol for further scaling-up studies. (T6.4, T7.1, T7.2, D3.10, and D6.4. Main Partners: AVE, iBET, AIT, and TCD).
- 4.2 **Implement of Life Cycle Analysis of the whole process** Means of verification: Final technologies for pretreatment and biodegradation will be selected in order to deliver the optimised energy and carbon efficiencies in accordance with lifecycle analysis (LCA). (T7.7. Main Partners: AVE, iBET, TCD, MLS, and IMGGE)



- 4.3 **Develop a strong dissemination and communication campaign.** Means of verification: Strong engagement, dissemination and communication campaign with the general public and industry, will serve to inform the wider community of the potential of BioICEP bioproducts and prepare for the transition from petroleum-based plastics. (T8.4 and D8.7. Main partners: AIT, AIM, AVE, MLS, and ACT)
- 4.4 **Business case development and commercialisation road-map** for the BioICEP technology including technologies such as enzyme cocktails and microbial consortia, as well as best route to market for selected bioproducts. *Means of verification:* Business plan recommending the go to market strategy for BioICEP technologies. (T8.5 and D8.9, Main Partners: AIT, MLS and AVE )

## 1.2. Relation to work programme

The EC has announced its commitment “to develop a new plastics economy” as a key priority of the circular economy action plan. The Commission’s main goals are to: 1) develop and promote more sustainable plastics and plastic products which fully respect reuse, repair, and recycling needs; and 2) curb plastic pollution and its adverse impact on our lives and the environment<sup>1</sup>. BioICEP is directly in line with the aims and can contribute to achieving the Commission’s target of an Energy Union with a modern, low-carbon, resource- and energy-efficient economy, the 2030 Sustainable Development Goals, and the Paris Agreement. BioICEP addresses all the CE-BioTEC-05-2019 requirements as mentioned in the following table.

CE-BIOTEC-05-2019 requirements:	How BioICEP addresses the requirements:
<i>The global market for plastics continues to grow due to their physical properties and benefits such as light weight, reduction of food waste, durability and cost. After being used, plastics should be separated in order to be subject to the most appropriate waste treatment processes. This is increasingly difficult and inefficient due to, for example, consumers' inaccurate identification of the appropriate types of plastics for recycling. Other plastic types, such as polystyrene, can even not be recycled if they have traces of food. Despite the worldwide efforts for degradation or recycling, large amounts of mixtures of plastics and other polymers end up in landfills or are used for the generation of energy. These methods lead to environmental contamination through the production of CO<sub>2</sub> or due to plastics reaching water courses and the sea where they persist and become toxic for the whole food chain.</i>	BioICEP technology is designed to create a circular economy for plastics waste and to provide a <b>cradle-to-cradle solution</b> to our current plastics waste challenge. Our groundbreaking objective is to develop technologies that mimic and operate in tandem with nature using <b>novel combinations of microbial and enzymatic digestion</b> of recalcitrant and degradable plastic waste. The project will funnel the resulting carbonaceous resources for the fermentation of new equivalent biopolymer plastics and bioproducts, thereby creating a circular plastic waste economy. Mixed waste streams will be processed which have <b>industry informed compositions</b> . These will be degraded through innovative combinations of techniques to overcome current recycling challenges, thereby demonstrating the potential to strongly contribute to the clean-up of the world’s plastic waste crisis. BioICEP can achieve high carbon-efficiencies and is a pertinent technology for our environmentally secure future.
<i>Novel biotechnological approaches should be applied for the sustainable biological degradation of mixtures of recalcitrant and degradable plastics.</i>	BioICEP technology proposes a <b>next generation biomimetic strategy</b> . Recalcitrant and degradable mixtures are subject to intensified enzymatic digestion followed by elevated microbial degradation delivered by a set of concerted novel booster technologies including: Pioneering <i>in situ</i> <b>biosensors to identify high performing strains</b> ; Innovative <b>high-throughput screening</b> expediting identification of the best performing enzyme ensembles; Tailored immobilization, stabilisation, and <b>directed gene evolution</b> generating highly efficient enzyme cocktails capable or operating at multiple stages of the degradation process; Targeted collection of the most evolved plastic degrading strains collected from global environmental pollution blackspots; and Designer strains with augmented activity via novel CRISPR-9cas technology.

<sup>1</sup> EU Action Plan for Circular economy, 2015; EU Circular Economy Package, 2018; EU Strategy for Plastics in the Circular Economy- Document 52018DC0028

CE-BIOTEC-05-2019 requirements:	How BioICEP addresses the requirements:  Associated with document Ref. Ares(2019)6080743 - 01/10/2019
	<p>BioICEP will create new <b>stable bacterial and fungal communities</b> with cutting-edge performance and capacities to cope with any potential toxic by-products from the process.</p>
<p><i>Proposals will develop environmentally friendly and sustainable solutions for managing the waste of plastics mixtures based on the use of communities of microorganisms with a set of complementary enzymes. The enzymes may be native or engineered using state of the art biotechnologies.</i></p>	<p>BioICEP's highly biomimetic design means that outcomes from the study will deliver an environmentally friendly and sustainable solution for managing mixed plastic waste streams. The aim is that the BioICEP technology is that outputs will gain <b>easy social acceptance</b> as it does not depend on or involve the adoption of new human behaviour around plastics.</p> <p><b>Communities of microorganisms</b> with a set of <b>complementary enzymes</b> are <b>core</b> to the BioICEP technology. Enzymes will be both native and engineered, derived from those identified by consortium partners and screened against those reported in the scientific literature for their ability to depolymerize each of the selected plastic and mixed plastic materials.</p>
<p><i>The microbial organisms will turn plastic mixtures into chemical constituents facilitating mineralisation, composting of otherwise recalcitrant and toxic polymers and facilitating production of high value products. Polymers such as polystyrene can also be included in the proposals.</i></p>	<p>Pre-treatment technologies used in the BioICEP study will include approaches that lead to greater plastic polymer surface roughness and hydrophilicity, in order to facilitate microbial colony attachment and accessibility of secreted extra-cellular enzymes to the polymer surfaces. Reduced MW and chain disrupted polymers can be processed through bacterial metabolism and biosynthesised into valuable products through metabolic pathways or mineralized. As part of BioICEP's strategy, microbial collection will be <b>targeted at some of the most polluted global sites</b> where microbes have advanced evolution for degradation of a wide range of waste plastics. A complete range of polymers found in mixed plastic waste will be included here, including polystyrene.</p>
<p><i>Proposals should:</i></p> <p><i>1) produce cocktails of enzymes using communities of microorganisms capable of degrading mixtures of biodegradable and currently non-biodegradable plastics into more basic chemical constituents;</i></p> <p><i>2) use a multidisciplinary approach based on biotechnology;</i></p> <p><i>3) create high value products and valorise mixed plastic waste.</i></p>	<p>1) The accelerated identification of novel enzymes, including those from the novel strains established in WP4 and WP5, will be carried out by new <b>fast track biosensing, genome sequencing, and tailored biochemical approaches</b>. Formation of plastics enzymatic cocktails and biocatalytic treatments to deliver monomers/monomer mixtures for valorisation will be carried out in WP3 using single and defined mixtures of emulsified polymeric material to establish the best performing mix of enzymes. Selected biocatalysts will further be improved using biocatalyst engineering strategies such as immobilization and directed evolution, as well as coupling to suitable indicators to generate efficient biosensors for expedited high performance enzyme discovery. 'Designer biocatalysts' will be developed to increase efficiency of depolymerisation of mixed plastic polymers.</p> <p>The best performing biocatalysts for depolymerisation of mixed plastic material will be <b>validated on the larger scale (10 L bioreactor)</b> and the obtained material from the depolymerisation of the pre-treated mixed plastic will be provided to WP6 for valorisation experiments.</p> <p>2) The BioICEP technology requires a <b>multidisciplinary approach</b> encompassing the convergence of diverse areas of expertise spanning <b>industrial polymer processing, microbial cultivation, enzymatic bioengineering, market knowledge, and environmental analysis</b>. Our consortium has been purposely selected to fulfil each of these areas of expertise while encompassing a capacity to collaborate fluidly with strong motivation, engagement, and dedication to the collective delivery of the project objectives.</p> <p>3) The conversion of the constituent molecules and monomers obtained from waste synthetic plastics degradation into value-added microbial products, namely, PHB/PHA, nanocellulose, and rhamnolipids will be carried out using microbial consortia and enzyme cocktails, developed and demonstrated in WP3, WP4, and WP5 for their ability to synthesize one or more of the envisaged products. Optimized bioprocesses protocols for</p>

CE-BIOTEC-05-2019 requirements:	How BioICEP addresses the requirements:
	<p>Associated with document Ref. Ares(2019)6080743 - 01/10/2019</p> <p>increased yields of high performance characteristic bioproducts for application in end-use products will be achieved using a feedback process with chemical, mechanical, and thermal analysis and WP 3, WP4 and WP5. In addition, in WP3 <b>microbial-cell-factory technology</b> which encompasses both polymer degradation capabilities and valuable product biosynthesis will be developed using systems biocatalysis approaches with potential to ultimately lead to highly integrated BioICEP technology platform attractive for industrial adoption.</p>

Table 2 - How BioICEP Addresses the Requirements

### 1.3. Concept and approach

#### 1.3.1. Analysis of the problem

Increased public concern and recent EU policies are driving a reinvigorated research endeavour to create a complete circular economy for plastic waste. As outlined in the European commission 'EUROPEAN STRATEGY FOR PLASTICS IN A CIRCULAR ECONOMY', the development of bio-based depolymerisation technologies and hence solutions to the world's plastic crisis is hampered by three main challenges: recalcitrant nature of plastics, non-biological degradability, and new technologies that must sustainably manage the constraints of highly plastic dependent lifestyles while addressing the global burgeoning plastic waste crisis in a low carbon footprint fashion.

#### Challenge 1: Factors hindering microbial/enzymatic polymer degradation

Microbes have a natural propensity to evolve to degrade new materials to maintain nature's cycle of generation, degradation, and regeneration. However a series of challenges are associated with this process:

- i. It is intrinsically slow and waste plastic substrates have only become prevalent in the past number of decades;
- ii. The current search for newly evolved plastics degrading strains has been extensive with only a limited number of strains discovered to date that have reasonable plastic degradation efficiencies;
- iii. Considerable and intensive scientific efforts are required to elucidate the complex underlying microbial and enzymatic degradation mechanisms that can be used to improve efficiencies;
- iv. Approaches to date have primarily focused on the development of individual strains and enzymes to degrade a specific plastic, rather than culturing mixed consortia and cocktails. *Pseudomonas*, which are ubiquitous in both aquatic and terrestrial environments are amongst the most cited for the degradation of a wide range of individual plastic to varying extents<sup>2</sup>. *Pseudomonas* species are attractive due to their diverse metabolic capabilities and genetic plasticity with strains engineered to oxidize aromatic, aliphatic, terpenic, and polyaromatic compounds;
- v. Microbial communities or mixed cultures with defined microbial strains are recognised as essential for successful high efficiency biodegradation of mixed plastic, in particular for polymers such as PE and PS that lack hydrolysable functional groups in their backbones<sup>3</sup>. There are often strong inhibiting factors preventing communities of microbial and fungal strains functioning in tandem to degrade plastics. In the case of mixed plastics, the presence of other easier to digest carbon sources, and toxic additives can act to inhibit strain performance; and
- vi. Enzymes such as esterases, lipases, and cutinases are hydrolases that are instrumental in plastic degradation. To date, typical production of these hydrolases is obtained by intracellular recombinant expression in species such as *E. Coli*, and is not conducive to scale-up for industrial purposes. Options such as methylotrophic yeast *P. pastoris* as hosts are more suited to simple production scale-up<sup>4</sup> with IMGGE, NTUA, and SDU having this expertise within the BioICEP Consortium.

#### Challenge 2: Biostability of Synthetic Plastics

The inhibiting factors of polymer degradation include: high hydrophobicity; low specific surface area, smooth surface topographies; extensive crystallinity; large inert molecular structures<sup>2</sup>; and low value-add of synthetic gas.

<sup>2</sup> Nikel, P. I. & de Lorenzo, V. *Metab. Eng.* **50**, 142–155 (2018).

<sup>3</sup> Wei, R. & Zimmermann, W. *Microb. Biotechnol.* **10**, 1308–1322 (2017).

<sup>4</sup> Gomerith, C. et al. *Front. Microbiol.* **8**, (2017).



- i. Synthetic polymer high MW and extensive repeating hydrophobic units render them insoluble in water prohibiting biofilm formation. PE, for example, a long-chain polymer saturated with ethylene bonds is highly hydrophobic. This restricts microorganism assimilation and effective enzyme adsorption and catalytic action;<sup>5,6</sup>
- ii. The low surface-to-volume ratio of plastic and plastic debris restricts microbial degradation. Increasing accessible surface area of PET by micronisation to obtain particle sizes less than 0.5 mm has been shown to improve subsequent degradation by bacterial polyester hydrolase enzymes;<sup>3</sup>
- iii. Polymer crystalline regions withstand microbial attack better than amorphous regions. Low-density PE (LDPE) is characterised by branching chains that prevent tight packing into a crystalline structure, while high density PE has little to no branching with the molecules stacking to form strong matrices, resulting in very slow degradation<sup>7</sup>. Although the crystalline parts of PET can be degraded by enzymes, this process is too slow for industrial biocatalytic recycling of PET beverage bottles or textile fibres<sup>8</sup>;
- iv. The high MW and lack of accessible carbonyl groups strongly impedes biodegradation. Polymers with hydrolysable chemical bonds in their backbone such as PET and PU are more susceptible than PE, PS, PP and PVC, which lack of hydrolysable functional groups.<sup>4</sup> Oxidation of the highly stable carbon-carbon (C-C) bonds, providing functional groups including carbonyl or alcohol groups and increasing hydrophilicity is a prerequisite to further depolymerization.<sup>2</sup> Under environmental conditions abiotic factors such as UV irradiation, oxygen, temperature, as well as the presence of chemical oxidants increase amenability to biodegradation over time; and
- v. The developing field of thermochemical depolymerisation of plastics suffers from a lack of added value as the focus lies mostly on liquid and gaseous fuel known as synthesis gas (syngas) or the re-polymerization to the original polymer. The application of waste plastics syngas as a feedstock for the microbial fermentation of biodegradable polymers is challenging and yields are low as solid forms are carbon are better feedstocks for these organisms<sup>9,10</sup>.

### Challenge 3: Human Plastic Dependent Lifestyles and the resultant indelible burden

The inventors of plastic, more than 100 years ago, could not have foreseen the full impact, both positive and negative, of future plastic products. **Plastics have permeated our lives, every corner of our planet and are now ubiquitous to human behaviour and terrestrial/marine ecosystems<sup>11</sup>.**

- i. Human manipulation of hydrocarbons has facilitated much of our social, technical, and economic advancement. Today it, would be very difficult to live plastic free, without injection moulded component-filled mobile phones and laptops, polyvinyl chloride bank and credit cards, plastic resin and composites, furniture, polyethylene packaged food and encapsulated pills, new high fashion acrylic jewellery, polyester clothing - the list is extensive. However, 90% of polled Europeans worry about the impact of plastic on the environment<sup>1</sup>. While we are witnessing a revolution in shopping culture, with reusable mugs for tea and coffee now trending and highly visible campaigns against plastic straws, we are acutely aware that these measures are woefully insignificant to adequately address global plastic consumption issues.
- ii. **Recycling as a method of plastic waste management is unlikely to hold the answer to sustainable or adequate plastic management** for an array of logistical, scientific, and economic reasons. Successful plastics recycling requires a multifaceted approach, which even if operating at high efficiencies, cannot fully answer the challenges of plastics waste management. The primary factors include the logistics and technical practicalities involved in collecting and sorting plastics which have overlapping densities over a very narrow range and the management of intrinsic human behaviour, such as our throw-away mentality and the realisation that for people to recycle their plastics it would need to be made very easy, with very little physical or mental effort required<sup>12,13</sup>

### Challenge 4: Difficulties in recycling mixed plastic waste sources

**Bio-based plastics are currently expensive** compared to petroleum-based plastics and are generally **synthesised from corn or other food crops**, which has adverse socio-economic implications of rising food

5 Krueger, M. C., Harms, H. & Schlosser, D. *Appl. Microbiol. Biotechnol.* **99**, 8857–8874 (2015).

6 Sammond, D. W. et al. *J. Biol. Chem.* **289**, 20960–20969 (2014).

7 Devi, R. et al. *Environ. Waste Manag.* (2015). doi:10.1201/b19243-13

8 Austin, H. P. et al. *Proc. Natl. Acad. Sci.* **201718804** (2018).

9 Heinrich, D. et al. *Appl. Environ. Microbiol.* **82**, 6132–6140 (2016).

10 Drzyzga, O. et al. *J. Chem. Technol. Biotechnol.* **90**, 1735–1751 (2015).

11 <https://www.economist.com/international/2018/03/03/the-known-unknowns-of-plastic-pollution>

12 Archer, K. *The Race Against Waste : Attitudes and Behaviours of Irish Consumers Towards Recycling and Segregation.* (2015).

13 Chow, C.-F., So, W.-M. W., Cheung, T.-Y. & Yeung, S.-K. D. *Plastic Waste Problem and Education for Plastic Waste Management.* in *Emerging Practices in Scholarship of Learning and Teaching in a Digital Era* 125–140 (Springer Singapore, 2017)

prices. Biodegradable materials have already entered the marketplace, for example, for use as food packaging. However both higher costs and poorer performance when compared to petroleum-based polymers such as PET and LDPE has resulted in low market penetration.

- i. Economic reasons dictate that **recycling only makes sense when clean material that is available in high quantities**. It depends on **advanced systems for collection and sorting of mixed waste streams** which is available in many developing countries. The price of plastic fluctuates with the price of oil and virgin plastics become far cheaper than recycled plastics when markets are depressed. Plastics cannot be perpetually recycled, unlike metals and glass even though they use much less energy to do so, since they degrade each time they are processed. Currently, the typical approach is to focus on high demand products such as plastic bottles, rather than plastics like yoghurt pots, margarine tubs, and plastic trays that are in low demand from manufacturers. The fact that recycled plastics rarely exhibit the equivalent mechanical properties to those of virgin plastics, as a result of polymer chain disruption during the recycling process, is an important contributing factor in the low European and global recycling success rates. While blended plastics, where virgin and recycled plastics are combined, and other approaches are used towards mitigating this challenge the fact remains that only a very small percentage of recycled bottles are used to make new bottles. Companies such as Coca-Cola report that only 7% of its plastic is from recycled material, while Nestle Waters North America uses just 6% recycled materials, which highlights the scale of this problem. The extra effort and expense required to separate plastics mean they often end up in landfill or are incinerated, with recycling rates falling rather than improving in many countries<sup>14</sup>. In the past quarter-century, “about 45% of plastic waste from throughout the world has been sent to China”. Mismanaged landfills in countries like China, Indonesia, the Philippines, Thailand, and Vietnam contribute to 60% of ocean plastic contamination. As of the 1<sup>st</sup> of January 2018, China has banned imports of unsorted plastic waste, which is forcing other countries to plan for the management of their own waste plastic.

### 1.3.2. BioICEP approach

#### 1.3.2.1. BioICEP Solution Concept

BioICEP merges:

- a. the best-in-class scientific recommendations across each of the diverse but relevant fields;
- b. selected cutting-edge approaches building on the considerable collective consortium expertise; and
- c. a series of new booster technologies designed to overcome the obstacles listed in challenges 1-4.

The BioICEP technology is designed to complete the life cycle for plastics that is derived from and operates in tandem with nature, ensuring an optimal premise for success, while greatly accelerating naturally occurring plastic degradation processes. BioICEP proposes a route for the seamless replacement of our current recalcitrant petroleum based plastics with equivalent biopolymers and bioproducts without disruption to human plastic dependent lifestyles and needs for examples in the demand that “*plastic prolong the shelf life of food resources which greatly reduce waste*”<sup>15</sup>.

In the BioICEP biomimetic strategy, mixed recalcitrant and degradable plastic waste undergoes a triple action depolymerisation process which includes: novel mechano-biochemical, enzymatic digestion and microbial degradation. Our team operates a series of cutting-edge technologies that will be collectively deployed, developed, and optimised to achieve the project objectives. Each of the processes used is mirrored by processes naturally occurring within the environment as indicated in Table 3, with a series of innovative approaches and novel techniques devised to accelerate and enhance the responses that would develop organically over much longer time scales.

Innovative & Novel Feature	Technological Approach
All green	Each process is selected to be highly environmentally sustainable and will be validated via LCA
Biomimetic	Each process has an equivalent approach in nature: Mechano-biochemical treatment equates to Environmental fragmentation Depolymerisation through enzymatic digestion mimics natural enzymatic activity Depolymerisation through microbial degradation mimics natural microbial activity

<sup>14</sup> <https://mitte.co/2018/07/18/truth-recycling-plastic/>

<sup>15</sup> Ayhan Z, Akademik Gıda 9(4) (2011) 36-41

Innovative & Novel Feature	Technological Approach
	Valorisation equates to regeneration Substitution of recalcitrant plastics equates to extinction and evolution
Fully integratable	The multiple processes are designed for their amenability for integration Novel technologies such as the planned new all-in-one degradation-synthesis platform will ultimately deliver a highly compact design
Bioplastic replacement of petroleum based plastics	Non-disruptive sustainable resolution of human destructive exploitation of natural resources.
Innovative new bioproducts	Biosynthesised products delivering new features surpassing conventionally produced products

Table 3 - Biomimetic solution based on the consolidation of interdisciplinary cutting edge technologies

A subsequent valorisation stage will utilise the harvested carbonaceous resources obtained from depolymerised mixed waste plastic feedstock for bioprocessing by defined microbial biosynthetic consortia. Successful utilisation of defined and natural consortia for PHA production from mixed carbon sources have previously been described<sup>16,17,18</sup>. Microbial consortia currently used to readily biosynthesis bioplastics and bioproducts using sugar and food waste feedstocks will be adapted for efficient bioprocessing using degraded waste plastic monomers and carbon constituents as the feedstock for bioproduct synthesis. New strains will be developed to upgrade and enhance existing communities. Strains with the capability to utilise plastic monomers and oligomers as a sole source of carbon and energy, which contain biosynthetic pathways for PHB or nanocellulose or biosurfactant synthesis will be identified or generated. In this manner, degraded polymer components such as monomers and oligomers will be recycled in a system that closely follows the providence of nature and enables the regeneration of new readily biodegradable plastics, creating a wholly circular plastic life cycle.

It is clear that a timely opportunity is presented to build upon buoyant public support to introduce and establish new ground-breaking technologies such as BioICEP to deliver a paradigm shift in the administration of plastic waste. Importantly, BioICEP fully addresses Challenges 1-4 above and without impacting on our high plastic dependencies or depending on improved recycling efficiencies and infrastructure. These challenges will be addressed through the meticulous and innovative research plan, presented here to surmount each of the limitations and impeding factors.

### Challenge 1 will be addressed through the use of enzymatic cocktails and microbial consortia overcome the existing limitations of using single microbial strains.

Stage two of our depolymerisation process is biocatalytic treatment. Here the emergence of hydrolytic enzymes from nature and advanced bioengineering including directed evolution will be used to expedite the development of enzymes deliver higher rates of depolymerization. We note that hydrolytic enzymes are already produced in bulk quantities and low cost for a variety of applications such as stain-removal agents in detergents and biomass depolymerization in second-generation biorefineries. In the search for improved biocatalysts, high-throughput screening methods jointly performed at both EU and Chinese partners, specifically designed to monitor activities are noted to be important to enable a rapid identification of these enzymes and their variants. Key booster technologies which fulfil this fast track function are shown in Table 4.

For the third stage of depolymerisation, formulation techniques and synthetic biology will be applied to engineer microbial communities for efficient degradation of mixed plastic waste. Microbial consortia have been used in biotechnology processes, including fermentation, waste treatment, and agriculture, for millennia as microbial consortia exhibit advantages over monocultures, including division of labour, spatial organization, and robustness to perturbations. Key state-of-the-art technological approaches combined with access to sites containing some of the worlds most intensively evolved strains will facilitate the formation of stable synthetic and natural microbial communities with high plastic waste degradation efficiencies.

<sup>16</sup> Coats, E. R., Watson, B. S. and Brinkman, C. K. *Water Research*, **106**, 26-40 (2016).

<sup>17</sup> Oliveira, C. S., Silva, C. E., Carvalho, G. and Reis, M. A. *New Biotechnol.*, **37**, 69-79 (2017).

<sup>18</sup> Nikodinovic, J., Kenny, S. T., Babu, R. P., Woods, T., Blau, W. J. and O'Connor, K. E. *Appl. Microbiol. Biotechnol.*, **80**, 665-673 (2008)


Booster Technologies	Action	Main Contributor
Proprietary mechano-biochemical approaches for MW reduction	Enhance  Waste reduction with document Ref. Ares(2019)168743 - 01/10/2019	EU iCOP
Novel biosensors for in situ degraded constituent detection	FastTrack high performance enzyme detection	China CAS
Stabilised enzyme sets for incorporation with pre-treatment	Augmented MW reduction	EU IMGGE/NTUA
Communities of stabilised bacteria and fungi for in tandem depolymerisation of mixed plastic substrates	Optimal depolymerisation of mixed plastics	EU and Chinese Entities
High performance genome editing for bioproduct production using CRISPR-Cas9 technology	Valorisation	China SDU
High performance bioplastics and bioproducts, including polymer blends and compatibilised polymer blends	Valorisation	EU iBET/AIT
Microbial platform with coupled all in one degradation-synthetic capabilities	Combined Depolymerisation & Valorisation	EU IMGGE

Table 4 - Booster technologies employed to deliver key actions and functionalities

**Challenge 2 will be overcome utilising a combination approaches where mechano-biochemical, enzymatic and microbial consortia treatments will result in >20% degradation mixed plastic waste.**

The BioICEP process commences with triple action polymerisation and the selection of mechano-biochemical techniques that correspond to an acceleration of the environmental conditions experienced by disposed waste plastics. Multifaceted combination technologies including ultrasonication, supercritical carbon dioxide (ScCO<sub>2</sub>) deep-eutectic solvent assisted depolymerisation, reactive extrusion, photo oxidation and blends with additives such as pro-oxidants and unsaturated polymers will be used to resolve hydrophobicity, high molecular weight, chemical and structural composition of petroleum based plastics that hinder their biodegradation.

There is a high degree of confidence within the scientific community that the current resistance of petroleum-based polymers to degradation can be circumvented by exploiting physico-chemical and microbial capabilities.<sup>2,4,6</sup> It is broadly accepted that synthetic biology has the potential to address these challenges and that the discovery of novel microbial strains and hydrolases, combined with the construction of highly active variants is key to the development of viable biodegradation technologies for post-consumer plastic waste<sup>3-5</sup>. Biostimulation has shown to be the most effective approach for the bioremediation of petroleum hydrocarbons contaminated soils using communities of microbes adapted to the existing physicochemical and environmental conditions, in particular to the prevailing pollutants<sup>19</sup>. Although hydrocarbon degraders include groups of Bacteria, Archaea, Fungi, and algae the number of surveys simultaneously studying the dynamics of microbial communities remain rare scarce<sup>14</sup>.

**Challenge 3 is addressed by the new high-performance bioproducts which are designed to provide viable, biodegradable alternatives to petroleum-based plastics.**

The BioICEP consortium's unique transcontinental biosynthesis capabilities will be engaged to deliver highly efficient production of new bioproducts and biopolymers which can be used as alternative petroleum based polymers. Both AIT and AIMPLAS have a great deal of expertise in developing novel polymer blends based on industry needs. This expertise will be leveraged in conjunction with other consortium members to develop of novel compatibilised plastics and bioplastic blends for industrial applications.

**Challenge 4 is addressed through the use of waste plastic as the feedstock for biopolymers.**

As such it removes any competition with food production, traditionally targeted as the feedstock for biopolymers. The strategy of the BioICEP business plan is to employ revenues from waste plastic degradation to offset any initial higher costs of the biopolymers produced compared to cheaper petroleum-based plastics. The costs associated with establishing BioICEP waste plastic regeneration are highly favourable compared to the current economic and environmental costs involved in recycling, storing, transporting, landfilling, and incinerating plastic waste. The commercialisation potential for the BioICEP technology is presented in the business plan in section 2.2.A.2. The BioICEP technology can be developed to high TRL post-project and launched as a profitable company providing sustainable solutions for the waste management sector and new bioproducts. There will also be lucrative options to licence a series of defined technologies to industries within the waste management sector and industries within the polymer value chain

<sup>19</sup> Siles, J. A. & Margesin, R. *Appl. Microbiol. Biotechnol.* **102**, 4409–4421 (2018).

### 1.3.2.2. Stages of the implementation

A highly considered and detailed research plan has been developed in order to surmount and address each of the limitations and impeding Challenges details in section 1.3.1 focusing on realising our vision of a sustainable avenue to a circular economy for plastics. This cross continental work programme will be delivered through close collaboration of both EU and Chinese partners as outlined in the Implementation section.

**WP2** focuses on an innovative combination of **multifaceted polymer MW reduction** and chain scission induction processes. these will be achieved through:

- Novel **proprietary ultrasonication-supercritical CO<sub>2</sub>-green chemical processes** that are designed to extract low MW compounds/oligomers from recalcitrant polymers, which is uniquely available through consortium member TCD.
- New combination mechano-biochemical approaches including reactive extrusion in combination with UV-degradation and deep-eutectic solvents.
- Pro-oxidant and degradation promoting additives

These methods will be used to strongly reduce polymer MW, form ample carbonyl groups, and provide good accessibility for subsequent enzymatic and microbial attack. To maximize the efficiency of pre-treatment processes, a combination of processes will be evaluated. In order to optimise the pre-treatment processes, samples will be characterized for their thermal, mechanical, chemical, and physical properties facilitating further refinement and optimization of the pre-treatment processes. The low MW oligomers and modified polymer compounds that are obtained during the pre-treatment processes will also be evaluated as compatibilisers to create compatible polymer blends suitable for industrial applications including the development of filaments for 3D printing which will build on previous work of AIT with Irish SME '[Shabra](#)' who have developed processes to produce structural beams from mixed plastic waste. The pre-treatment techniques will be evaluated to determine their capacity to enhance the bio-degradation of individual and mixed plastic waste. Hence, the pre-treated, mixed plastics, produced using the best performing and most sustainable processes will be granulated, shredded into micro pieces or ground into powders to provide feedstock for supply to WP3, WP4, and WP5.

In the case of biocatalytic digestion as outlined in Figure 1, **WP3** is dedicated to the:

- The **development and formation of plastics enzymatic and biocatalytic treatments** to deliver monomers/monomer mixtures for valorisation.
- **Cocktails of enzymes** that can be used at various stages of pre-treatment and after pre-treatment of mixed plastic waste will be developed using novel and innovative screening strategies.
- **New high-throughput screening** technology and the development of novel high performance strain biosensors for expedited advancement of single and mixed plastics degradation.
- Biocatalyst improvement using engineering approaches, formulations, and the generation of all-in-one platforms with **simultaneous high degradation and biopolymer synthetic performance** that will be supplied to in WP6 for valorisation.

This work package will commence by screening and identifying the most suitable enzymes for depolymerisation of defined mixture and real plastic waste (IMGGE, NTUA, SDU). Standard enzymatic activity evaluation procedures will be adopted and optimised by using i) emulsified polymeric material, ii) synthetic model compounds, and iii) defined mixtures thereof as substrates for defining the best performing enzymes against single substrates, as well as the best performing mix of enzymes (IMGGE, NTUA, SDU). Selected biocatalysts will further be improved using standard biocatalyst engineering strategies such as immobilization and directed evolution. In addition, work will be done for the establishment of improved assays for biodegradation of plastic materials encompassing synthesis of suitable model compounds to represent each plastic material, as well as coupling them to a suitable indicator to generate efficient biosensors (CAS, IMGGE). Both strategies will be validated within this work package, as well as within microbial and consortia screenings (WP4 and WP5).



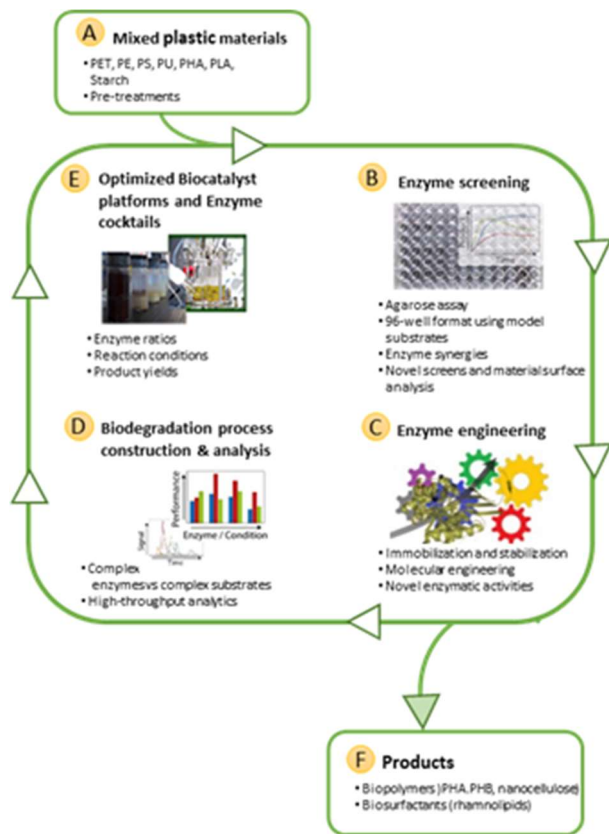


Figure 2 - Schematic of biocatalytic and enzymatic cocktail development for mixed plastics degradation

Novel enzymatic activities will be identified from the novel **Enzyme screening** (WP4) to **boosting plastics degradation capacity through genome sequencing and standard biochemical approaches**. 'Designer biocatalyst' will be developed to:

- increase the efficiency of depolymerisation of mixed plastic polymers
- encompass polymer degradation capabilities with valuable product biosynthesis using systems biocatalysis approach.

The best performing biocatalysts for depolymerisation of mixed plastic material will be validated on the larger scale (10 L bioreactor, WP6) and the obtained material from the depolymerisation of the pre-treated mixed plastic material will be provided to WP6 for valorisation experiments. In the case of **microbial consortia degradation, biodiversity screening of consortium partner's existing biobank and newly isolated strains**, will be undertaken initially using individual plastics. Sources of plastic waste from highly polluted global sites including China and Serbia, will be used to isolate new strains using conventional approaches, the iCHIP method and new accelerated screening methods. The selected microbes will be characterised by Chinese and European partners for their ability to degrade targeted

plastics after pre-treatment, depolymerisation enzyme activities, providing an information feedback loop to WP2, WP5, and WP6. The best strains from all screens will be used to identify and isolate novel enzymatic activities (WP3) and to **create a defined consortia** in WP5, which **can breakdown mixed plastic**. 'Designer strains' will be generated to boost plastic hydrolysing capacities based on microbial host platforms such as *Streptomyces lividans* and *Pichia pastoris*. In addition the identified degraders will be screened by all partners for PHB, rhamnolipid, and nanocellulose production potential and fed into to WP6.

WP5 will **develop stable microbial communities or defined mixes** of microbial strains with improved performance in comparison to whole cells or single strains. These will be applied at various stages of biological degradation, as well as at the valorisation stages. This will be achieved in close collaboration with WP3 and WP4. Established communities will be validated in both mixed plastic degradation, as well as in valorisation experiments (WP6). Standard formulation techniques for the development of mixed microbial inoculants will be applied, as well as principles of synthetic biology in order to engineer microbial communities for efficient mixed plastic biotechnological recycling process. Communities will be monitored during the process using a proprietary bioinformatics pipeline.

In the valorisation stage, WP6 is dedicated to the **conversion of the constituent molecules** and monomers obtained from waste synthetic plastics degradation **into value-added microbial synthesised products**, namely, PHB, nanocellulose, and rhamnolipids. Different microbial strains and consortia, developed and demonstrated in WP3, W4 and WP5 for their ability to synthesize one or more of the envisaged products will be used to develop and optimize bioprocesses for their high yield production in bioreactor experiments. The bioproduction as well as the downstream processes will be optimized at laboratory scale using advanced monitoring techniques and metabolic modelling. Extensive testing, processing, and analysis of the bioproducts will be conducted for products in applications such as rigid and flexible food packaging. This will facilitate a feedback process to allow the optimisation of the fermentation process for the enhancement of the bioproducts and will enable improvement of the processing quality and integrity. Data for a preliminary cost assessment for each product will be provided.

WP7, **aims to provide a prototype production plant using a 50 L-100 L pilot** for the BioICEP process. This integrated prototype system will include a modular biocatalytic and microbial pretreated plastics degradation bioreactor, biomass separation and bioproduct fermentation operated in accordance with the parameters

developed in WPs 2-6, including the implementation of process controls and automatization. The generation of PHB/rhamnolipid/nanocellulose bioproducts will be optimised and fully characterised to ensure their potential for end of use applications. The pilot will be designed for the operation with non-genetically modified microorganisms and the setup will be constructed at AVE's tech hall (Ghent, Belgium). Life cycle analysis will be carried out in conjunction with TCD in order to establish the environment impacts of each stage of the technology. This pilot plant will be non-GMO operated, however in the future GMO plants can be operated without risk of release of GMO contamination.

Building on the business plan detailed in section 2.2.A.2 a **fully developed business plan** will be prepared, **demonstrating the BioICEP cost efficient biotransformation of waste plastics into bioproducts** with high performance properties for applications including food packaging cosmetics, pharmaceuticals, and 3D printing. Following the successful completion of this project, further development to higher TRLs will enable the full capabilities of the BioICEP technology concept to be optimally exploited at industrial level. Hence each of the processes will be selected and developed towards the ultimate target of operation under industrial conditions. The combination of these factors means that BioICEP presents a highly lucrative business case while achieving high carbon efficiencies making it a pertinent technology for our environmentally secure future.

### 1.3.3. Positioning of the project

The overarching purpose of BioICEP is to bring an integrated waste plastics biodegradation and bioproduct production technology from TRL 3 to TRL5/6. The starting and final technological readiness level of each of the pan EU-China technologies components and implementation targets during this project are provided in Table 5.

Technology Description:	Activities Description:	TRL Start	TRL End
Mechano-biochemical approaches	Proprietary and new combination mechano-biochemical technology development	2-4	5
Accelerated development of stable defined microbial consortia	Targeted Screening, biobank enrichment, Strain engineering	3	5
Accelerated development of high efficiency.enzyme cocktails	Immobilisation and directed evolution. Novel biosensors for in situ degraded polymer constituent, High performance genome editing for bioproduct production using CRISPR-Cas9 technology	3	5
Stabilised enzyme cocktails integration into depolymerisation I) process.	Multiple approaches including crosslinking to achieve increased stability, reusability and cost reduction.	2-4	5
Depolymerised waste plastic as novel fermentable carbon feedstock	Optimisation of 3 stage depolymerisation process	2	5
High yield biosynthesised products: PHBs, nanocellulose and rhamnolipids	Metabolic modelling and feedback loop based on extensive testing, processing, analysis and comparative testing with current on the market equivalent products	4	5
High performance bioplastics and bioproducts	Characterisation and Processing feedback information loop and polymer blends and compatabilised polymer blend development	4	5
Microbial platform with coupled all in one degradation-synthetic capabilities	Microbial-cell-factory technology development	2	5
Fully integratable design	Integrated assemble of multiple processes. Development of novel technologies such as new all-in-one degradation-synthesis platform will ultimately deliver a high compact design	2-3	5
Modular Pilot Plant	Demonstration of all in one mixed plastic waste depolymerisation and biosynthesis of high value bioproducts	2	5-6
High sustainable multiprocess	LCA Validation of low environmental impact	3	5
Business plan preparation	Business model development of BioICEP cost efficient biotransformation of waste plastics into bioproducts	2	5



Technology Description:	Activities Description:	TRL	TRL
		Start	End
Bioplastic replacement of petroleum based plastics demonstrations	Sample of both processed food packaging using PHB, nanocellulose and blends as well as 3D printable bioplastics will be provided for assessment by industry.	3	5
Innovative new bioproducts	New biosynthesised surfactants rhamnolipids	3	5
Preparation of Industry players for transition from petroleum based plastics	Industry engagement and dissemination	4	5

Table 5 - Technology TRL progression during the BioICEP project

### 1.3.4. National or international research and innovation activities linked with the project

The consortium has ran in the past several related national or international research and innovation activities in related fields with BioICEP project. The knowledge and the results developed in these project will be linked with BioICEP as mentioned in the following table.

Partner	Project Name:	Type of Project:	Results linked with the Project:
AIM	LIFE EXTRUCLEAN - Hazardous substance removal during the recycling of polyethylene waste packaging CLIPP+	National; (LIFE13 ENV/ES/000067)	scCO <sub>2</sub> system to be applied on materials to be recycled
	ENZOX2	EU; H2020 GA 673663	scCO <sub>2</sub> system to be applied on recycled materials to be decontaminated
	ENZOX2	EU; H2020 GA 720297	Application of enzymes and biotechnology to develop bioplastics
AIT	PROTECTIVE	EU; H2020 GA 700071	Knowledge Exchange
	Eco-MixPlas	National; CF3002	Mixed Plastics Waste as a Valuable Resource: High value new products from mixed plastics waste
	STRUCCO	National; CF2315	Structural Thermoplastic composites from recycled PET
AVE, iBET	YPACK	EU; H2020-SFS-2017-1	Development of high performance polyhydroxyalkanoates based packaging
	MicroNOD	National; MIP- ICON project, Flanders Cleantech research for transition	Microbial immobilization and microbial communities analysis
	ROBANODE	EU; FP7	Sonochemically assisted materials preparation; surface modification and characterization of plastics
IMGGE	The upcycling of waste plastic packaging material to a biodegradable plastic	EU; Green Innovation Vouchers Scheme for Serbia (EBRD)	Biotechnological conversion of the PE waste material into PHA
	Microbial diversity study and characterization of beneficial environmental microorganisms	National; Grant No 173048	Microbial biobanks
iBET	RES URBIS	EU; H2020-CIRC-2016 OneStage – 730349	Production do PHA from urban bio-waste
MLS	BE-BASIC projects	National; Belgium	Microbial biobanks; white-rot fungi enzymes
LIT	Irish Bioeconomy Foundation	National, Enterprise Ireland	Microbial biobanks; conversion of waste sources to valuable materials
	BAMMBO	EU; FP7 (265896)	Microbial biobanks and screening of microorganisms
NTUA	OPTIBIOCAT	EU, FP7 KBBE.2013.3.3-04	Optimized esterase biocatalysts for cost-effective industrial production
	TASCMAR	EU; H2020 634674	Discovery of novel enzymes for biocatalysis
	NoWasteBioTech	National; Hellenic Foundation for Research & Innovation	Conversion technologies of waste biomass to valuable chemicals
TCD	AMBER	National; Science Foundation Ireland, with co-funding from 38 industry partners	Materials research and technologies of treatment and characterization
	SYNPOL:	EU-FP7 project; 311815.	Platform integrating biopolymer production through modern processing technologies, with bacterial

Partner	Project Name:	Type of Project:	Results linked with the Project:
			Associated with document Ref. Ares(2019)6080743 - 01/10/2019
	Agrichemwhey:	H2020; grant agreement No :74431020	fermentation of syngas, and the pyrolysis of highly complex biowaste. Convert dairy sidestreams into added-value products – specifically L-Lactic acid, polylactic acid, minerals for human nutrition and bio-based fertiliser.
CAS	CAREER	National; Chinese National Science Foundation (Award ID 0644678)	Protein engineering techniques; Recombinant microorganisms
	Design and application of high-throughput screening tools: a review	National; Ministry of Science and Technology of China Grant 2013CB734003	High-throughput screening tools
SDU	Engineering Corynebacterium glutamicum	National; National Natural Science Foundation of China (31370085)	Microbial strain engineering techniques; Synthetic Biology approaches
	National Basic Research Program of China	National; National Basic Research Program of China, 2007CB707803	Recombinant strains for PHA production

Table 6 - Consortium national and international research and innovation activities related to BioICEP

### 1.3.5. Describe how sex and/or gender analysis into account in the project's content

The gender dimension of this research has been analysed and is found to be largely neutral. This is due to the fact that plastics usage is universal and research in this and related fields are not gender specific. However, It is predominantly females in the household who are decision makers for purchasing packaged goods. Hence having a strong female consortium leader will add credence to targeted dissemination towards this cohort. The technologies, processes and products developed in BioICEP are gender-independent. Provisions are in place to ensure that gender is part of the research design and systematically controlled for throughout the planned research process.

Monitoring the **progress toward gender equality in science has become a well-established activity** of the European Union research policy. In accordance with these principles, the approach of BioICEP is fully in line with Directive 2002/73/EC of the European Parliament and of the Council of 23 September 2002 on the implementation of the principle of equal treatment for men and women as regards access to employment, vocational training and promotion, and working conditions.

The BioICEP consortium has a **female coordinator which is an under-represented gender in engineering**. All of the BioICEP Partners have internal gender equality jobs creation and promotion policies. The consortium will foster the presence of women in the technical and management teams and on decision boards implemented to govern the BioICEP project.

BioICEP aims to contribute to these policies for enhancing Gender Equality and has numerous female participants (see section 4) in the project and actively encourages female science and industrial experts to play leading roles in work packages and tasks. To this end, the project coordinator is female, 50 % of the WP's are female led, one third for the Chinese participants is female led and 42% of the EU participants are female. AIT has a female EU-China engagement officer, which has been instrumental in building the strong EU-China relationship demonstrated within this consortium.

Participant:	AIT	ACT	AIM	AVE	CUT	IBET	IMGGE	LIT	LOG	MLS	NTUA	TCD	BIT	CAS	SDU
Gender:	Female Coordinator	Male	Female WPL	Male WPL	Male	Female WPL	Female WPL	Male WPL	Female	Male WPL	Male	Male WPL	Male	Female	Male

Table 7 - Gender analysis of the BioICEP consortium (WPL: Work Package Leader)

## 1.4. Ambition

### 1.4.1. Current state of the art

The field of plastics depolymerization suffers to date from a lack of added value as the focus is largely on synthesis gas (syngas) production, re-polymerization to the original polymer. The application of waste plastics syngas as a feedstock for the microbial fermentation of biodegradable polymers is challenging and yields are low. Bio-based plastics are currently expensive compared to petroleum-based plastics and are generally synthesised from corn or other food crops, which has adverse socio-economic implications.

		GHG Emissions & Carbon Footprint	Energy Efficiency	Efficient resource use	Cost Efficiency	Valorisation and Bioproduction
Emergent	Gasification	lower than conventional fuels	Medium	Largely cradle to grave	Medium return on investment	Syngas for energy Limited bioprocessing capacity.

Emergent	Biomass	Cultivation/processing leads to GHG emissions	Medium	Can compete with food production	Petroleum plastics less expensive	Medium quality bioproduct e.g. PHA packaging with low industrial uptake
Current	Recycling & Repolymerisation	Medium	Medium	Low recycling rates Low levels of repolymerisation	Poor return on investment	Product depreciation with each cycle. Low industrial uptake
Current	Incineration & landfill	High	Poor	Poor	Annual cost of \$139bn	Generates Pollution
Next Generation	BioICEP	Low	Excellent	Excellent	High potential return on investment	High performance bioproduct quality

Table 8 - Current state of the art and emergent approaches to plastic waste management compared to the proposed BioICEP technology

#### 1.4.2. Patent and related funded projects

In January 2018, the European Commission took a big decision to combat plastic pollutions by adopting the *European strategy for plastics in a circular economy*. The Plastic Strategy has introduced new Horizon 2020 funding opportunities resulting in several forthcoming calls: sustainable solutions for bio-based plastics on land and sea (H2020-BG-2018-2020) and raw materials innovation for sustainable processing, reuse and recycling (H2020-SC5-2018-019-2020). Both are calls for innovation action, with a focus on sustainable strategies for (bio)plastic reuse and recycle. Similarly, many current projects are focused on developing bioplastic alternatives for the existing petroleum-based plastics, such as GloPack (H2020, grant agreement n° 773375), MyPack (H2020, grant agreement n° 774265) and YPACK (H2020, grant agreement n° 773872). In comparison, the current project is aimed at simultaneously biodegrading and valorising plastic waste products via enzyme cocktails and a consortia of microorganisms and producing high value microbial end products (e.g. PHB/rhamnolipids/nanocellulose).

Past research into plastic degradation have yielded interesting patent applications, which could be compatible with the current proposed development. For example, US patent 2015/0203666A1 (appl. status: Active) describes the use of an additive during the manufacturing of plastics to cause biodegradation at a predetermined time. The patent application WO2014/067081A1 (appl. status: Active) uses insects capable of degrading petroleum-based plastics and microbial consortia to degrade waste plastics. End products are not well defined consisting of biomass to be used as a source of lipids, fatty acids, proteins and chitin for potential industrial products such as biofuels, emulsifiers, surfactants, lubricants and flocculants.

Patents exist that relate to the microbial production of rhamnolipids using genetically modified organisms (EP2573172A1, appl. status: Withdrawn) and the use of biosurfactants produced by *Pseudomonas* sp., including rhamnolipids, as a biological control agent (US2011/0306569A1, appl. status: Abandoned and 5,767,090, appl. status: Expired). Bacterial cellulose production has also gained extensive attention due to its economic value. WO2005/003366A1 (appl. status: Active) relates to the synthesis of cellulose by *Acetobacter xylinum* and its application as wound dressing. WO2012/021056A1 (appl. status: Active) discloses a pretreatment method to prepare biomass to facilitate microcrystalline production from palm oil milling waste.

Regarding the microbial production of polyhydroxyalkanoates (PHA), US9243266B2 (appl. status: Active) embodies the microbial production of PHA from volatile organic compounds, WO2002/008428A2 (appl. status: Active) relates to the production of PHA from alcohol and sugars by recombinant *Escherichia coli* and CN102206596A involves the production of PHA by *Pseudomonas lundensis*, grown on glucose.

In the **BioICEP project**, the research will be **distinct from the patent protected** procedures in **terms of substrate composition, substrate treatment with novel mechano-biochemical processes**, the use of **mixed cultures** of bacteria (as opposed to pure cultures) and **novel enzyme cocktails**. Further differentiating factors include the fact that BioICEP microbial consortia will contain different bacteria and will also contain **fungi**. This in combination with the use of enzyme extracts to aid plastic degradation in conjunction with mechanical methods is a novel process. The BioICEP **end products are clearly defined** and will include Polyhydroxybutyrate (PHB) and nanocellulose for compostable food packaging applications, compatibilised bioplastic blends for the production of 3D printing filaments, and rhamnolipid biosurfactants for the cosmetic and pharmaceutical industries.

#### 1.4.3. Progress beyond the state of the art and innovation potential

This project is ambitious in that it aims to take existing mixed recalcitrant plastics and break them down into simpler products. In this project, plastic degradation will be accelerated by using booster technologies such as proprietary mechano-biochemical processes, the use of novel biosensors for analysis of in situ degraded

polymer constituent and establishment of enzyme and microbial consortia. Bioproduct production will be enhanced using technologies such as **genome editing and optimization of microbial fermentation** resulting ultimately in a microbial platform with coupled all in one plastic degradation-synthetic capabilities.

Approaches to date have primarily focused on the development of individual strains and enzymes to degrade a specific plastic, rather than culturing mixed consortia and cocktails or indeed mixed plastic substrates. Further, this project has an additional focus on the conversion of the compounds, liberated during depolymerisation of plastics, into high value end products using proprietary mechano-biochemical processes. The end-products from these processes can then be converted into bioplastics and useful biomolecules using microbial fermentation. **No other projects to date have taken a nature inspired all-inclusive strategy to target plastic pollution** resulting in the production of bioplastics bioproducts enabling a new plastic waste based circular economy.

The BioICEP technology will lead to a series of technical innovations with significant environmental and economical benefits. Each of these innovations require multiple partner inputs including novel technologies spanning the EU-China consortium.

Innovation & Lead Participant	Novel Technology	Selected novel technologies used for development	Target Market
Innovation 1 12 TCD, Ireland 1 AIT, Ireland	Novel Mechano-biochemical Mixed plastic MW reduction	Innovative sustainable combinations including proprietary ultrasonication-supercritical CO <sub>2</sub> -green chemical process	Licencing to multiple waste management companies
Innovation 2 6 IMGGE Serbia 14 CAS China	Depolymerising Enzymatic Cocktails	Designer enzyme engineering Novel biosensors for in situ degraded polymer constituent detection	Licencing to multiple waste management companies and/or
Innovation 3 10 MLS Netherlands 13 BIT China	Depolymerising Microbial Consortia	Targeted screening and biobank enrichment	Licencing to multiple waste management companies
Innovation 4 7 iBET, Portugal 15 SDU China	Bioprocessing of degraded mixed plastics	Biosynthesis using fermentable carbon from mixed plastics CRISPR-Cas9 genome editing	Licencing to bioplastics manufacturers
Innovation 5 All partners	High performance Bioproducts	PHB and nanocellulose e.g. for food packaging. Bioplastic filaments for 3D printing, Rhamnolipid biosurfactants	Licensing and direct sales
Innovation 6 6 IMGGE Serbia 11 NTUA Greece	Ultimate all-in-one degradation biosynthesis	Microbial platform with coupled all in one degradation-synthetic capabilities	highly compact plant design for commercialisation

Table 9 - BioICEP technology innovations and the contributors of key novel booster technology enablers

The project will **culminate with a modular demonstrator pilot plant** development which will integrate these innovations to demonstrate the implementation and the high potential of the BioICEP technology as a pertinent route to a circular economy for plastics efficiency. The all-in-one BioICEP pilot plant will encompass plastic degradation-fermentable carbon harvesting and bioproduct synthesis and will be constructed in AVE's industrial bioreactor facility in Ghent. The process will be validated by LCA as highly environmentally sustainable. Alternatives to petroleum based plastics will be developed and innovative new bioplastics and bioproducts will be created surpassing conventionally produced bioplastic products.

The BioICEP business plan foresees the employment of revenues from waste plastic degradation to offset any initial higher costs of the biopolymers produced compared to cheaper petroleum-based plastics. Thus, the BioICEP project has the potential to evolve into a waste management and/or product manufacturing company as detailed in the business plan outlined in section 2.2.A.2. During the course of the project, different business models, markets and distribution channels will be considered. Furthermore, the experimental work will select and optimize a microbial production system for one of the predetermined high value end-products: PHB / rhamnolipid / nanocellulose. The BioICEP market-driven approach can therefore deliver an important contribution to the EU Plastic Strategy and the UN SDGs.

END OF SECTION 1

# SECTION 2 - IMPACT FRONT PAGE PLACEHOLDER

12 key actions will result from the successful delivery of the BioICEP project, as follows:

BioICEP will:

1. **Develop** at least FOUR improved enzymatic activities enabling the degradation of plastics mixtures (such as PETase, PHA-depolymerase, Cutinase, Triple active biocatalyst);
2. **Enable** the degradation of at least TWENTY percent of non-biodegradable plastics found in plastic mixtures which will be demonstrated by percentage weight loss post depolymerisation;
3. **Identify** at least FOUR high performance, high added value, high growth potential products that will be sustainably produced from waste plastic mixtures (such as Bioplastics for Flexible food packaging, Bioplastics for Rigid food packaging, 3D printable Bioplastic Filaments and Biosurfactants);
4. **Deliver** ONE sustainable and environmentally friendly 100L pilot plant for the integrated degradation of waste plastic mixtures and production of bioproducts;
5. **Organise** ONE international conference, TWO workshops and attend at least SIXTEEN international conferences and FOUR trade fairs to enable cross-border and cross-continent stakeholder engagement;
6. **Enter into** direct contact with at least TWO HUNDRED international companies by using the consortium's 600+ specialist company network to prepare and enable them to transition from petroleum based plastics;
7. **Create** ONE spin-out company in 2021 to commercialise the project's expected innovative processes, products and services;
8. **Negotiate** SIXTEEN evaluation licenses, four of which will be with the consortium's SME partners, to validate the high value bioplastics market potential and develop new market opportunities;
9. **Provide** a route to company cost savings of FIVE to FIFTEEN percent, enable a TEN fold job creation increase potential and a TWO fold increase potential in innovative products to bioplastics manufacturing companies by demonstrating an alternative to biomass;
10. **Give** plastics value chain companies a solution to cut their GHG emissions by TEN to TWENTY FIVE percent and waste management companies a solution to cut their GHG emissions by THIRTY to EIGHTY percent;
11. **Offer** TEN short-term internship and secondment opportunities for researchers to maximise the technology transfer between the consortium partners and also to enhance EU-China cooperation activities;
12. **Contribute to** TWELVE out of the seventeen UN Sustainable Development Goals.

Figure 3 - BioICEP Expected Impacts

### 2.1.1. Expected Impacts mentioned in the work-programme

**IMPACT 1 - A combination of microorganisms expressing at least three novel or improved enzymatic activities enabling the degradation of mixtures of plastics**

**BioICEP will develop at least FOUR improved enzymatic activities enabling the degradation of plastics mixtures (such as PETase, PHA-depolymerase, Cutinase, Triple active biocatalyst).**

**#1**

The isolation of novel strains and microbial communities using BioICEP's underlying iCHIP technology introduced in Section 1.1. in combination with high throughput assays, will fast-track the detection of novel enzymatic activities applicable to the biotechnological degradation of mixed plastics.

The new enzymatic activities will be developed using genome analysis of up to five strains coupled with chromatographic protein isolation and analysis. The directed evolution of PETase, PHA-depolymerase, and broader substrate scope and cutinase for improved activity will be conducted see Table 10. In addition, microbial cell surface display strategy, i.e. accord of enzymes to be exhibited on the surface of cells by fusing the proteins of interest with the anchoring motifs, will be carried out using *E. coli* and/or *Pichia* as hosts to yield at least three activities per single biocatalyst.

No.	Improved Enzyme	Novelty for improved activity against mixed plastic substrates
1	PETase	Increased PET digestibility; higher resistance against products of digestion
2	PHA-depolymerase	Broader PHA substrate scope including short and long C-chain digestion
3	Cutinase	Multiple substrates including PE, PET and PHA; increased activity
4	Triple active biocatalyst	Simultaneous high activity against multiple mixed plastic substrates PE, PET, PU

Table 10 - Enzymes with improved activities enabling the degradation of different plastics

These improved enzymes will form part of a cocktail to work in tandem with mechano-biochemical pre-treatment and microbial consortia post-treatment enabling efficient digestion of plastic mixtures.



## IMPACT 2 - Degradation of at least 20 percent of non-biodegradable plastics found in plastic mixtures

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The minimum expected degradation of mixed waste plastics on completion of the project is **20.5 ±0.5 %** in the case of recalcitrant plastic components and will be measured by % **weight loss post depolymerisation**.

#2



The expected degradation impact of the BioICEP technology, which includes a combination of microorganisms expressing at least three novel or improved enzymatic activities when applied to a typical commercial mixture of plastics, is detailed in Table 11.

Plastic	% Mixture (by weight)	Indicator: % degraded post Depolymerisation I + II (by weight)	Indicator: % degraded post Depolymerisation I + II +III (by weight)
Polyethylene (PE)	29±3	2.9±0.5	3.7±0.5
Polypropylene (PP)	19±3	0.95±0.5	1.2±0.5
Polyvinyl Chloride (PVC)	12±2	0.6±0.2	1.1±0.5
Polystyrene (PS)	8±2	1.6±0.5	2.5±0.5
Polyethylene Terephthalate (PET)	6±1	3±0.5	5.2±1
Polyurethane (PU)	7±1	2.1±0.5	3±1
Polylactic acid (PLA)	1±0.5	0.7±0.2	1±0.3
Starch	1±0.5	1±0.3	1±0.3
Additives & Miscellaneous	17±3	NA	NA
Total Mixed Plastics % (incl. additives etc.)	100	15.5±2.5	22.5±3
Total Recalcitrant Mixed Plastics % (excl. additives etc.)	98	13.8±2	20.5±3

Table 11 - Expected degradation percentages for specific plastics within mixtures that are typically dispatched for landfill or incineration

The estimated percentage of each type of plastic, both recalcitrant and biodegradable, contained within a typical commercial plastic mixture is given in column 1 of Table 11. Note that such typical plastic mixtures also contain approximately 17 % of spurious and miscellaneous items as well as commercially added additives which are omitted from the calculation. The degradation by weight following stage II and stage III of the BioICEP depolymerisations are listed in columns 2 and 3 of Table 11. These expected values are conservative compared with some literature reported values and are expected to occur within 2-4 weeks and 4-8 weeks respectively. These values have been compiled based on the considerable cumulative expertise of the consortium partners. The breakdown of the waste as indicated here is based on work carried out by Consortium leaders with Irish waste reproducers (Shabra) once high value PET and HDPE has been removed for recycling. This waste typically is incinerated or landfilled as it is not economical to sort. A more detailed investigation into the variation in mixed waste plastic constituent plastics will be carried out in collaboration with the consortium industrial partners in WP1.

## IMPACT 3 - At least two high-added-value products sustainably produced from plastic mixtures

BioICEP will identify at least **FOUR high performance, high added value, high growth potential products** that will be sustainably produced from waste plastic mixtures (such as Bioplastics for Flexible food packaging, Bioplastics for Rigid food packaging, 3D printable Bioplastic Filaments and Biosurfactants).

#3

BioICEP bioplastic and bioproducts are targeted towards high-need, high-growth market segments. The bioplastics are expected to deliver high-performance levels for consumer products, surpassing those currently achievable using contemporary biosynthesised plastics such as PHA, as detailed in Table 11. Packaging remains the largest field of application for bioplastics with almost 65 percent (1.2 million tonnes) of the total bioplastics market in 2018.<sup>20</sup>

The rapidly expanding 3D printing market, which is predicted to reach USD 34.8 Billion by 2024 at a CAGR of 23.25%,<sup>21</sup> requires new bioplastic based resins and filaments. The third BioICEP high value added product is targeted at the filament segment, which in 2018 accounted for the largest share of the 3D printing plastics

20 European Bioplastics, Bioplastics market data 2018, Global production capacities of bioplastics 2018-2023, p. 3

21 [https://www.marketsandmarkets.com/Market-Reports/3d-printing-market-1276.html?gclid=Cj0KCQIAtvPjBRDPARIsAJfZz0qmuYHgvEaNsogEknczEHH8qefa-Ru5TNMF44BJGFA26UrsYtnAffwaAv1QEALw\\_wcB](https://www.marketsandmarkets.com/Market-Reports/3d-printing-market-1276.html?gclid=Cj0KCQIAtvPjBRDPARIsAJfZz0qmuYHgvEaNsogEknczEHH8qefa-Ru5TNMF44BJGFA26UrsYtnAffwaAv1QEALw_wcB)



market and is projected to dominate the market by 2023. Rhamnolipids are the fourth target bioproduct of the BioICEP project and are the growth leading product with the global biosurfactants market estimated at USD 4.20 Billion in 2017 and projected to reach USD 5.52 Billion by 2022, at a CAGR of 5.6 % from 2017 to 2022.<sup>22</sup>

Table 12 defines the bioplastic and biosurfactant user product needs that will be met upon completion of the project:

Bioplastic products	Users	Defined needs to be met upon completion of the project
1 Bioplastic for Flexible food packaging	Food packaging Industry	Flexible food packaging biosynthesised PHB and nanocellulose biodegradable polymer/ polymer blend sample films with equivalent properties to current PE packaging films carried out in collaboration with AIT and client companies. PHB is compostable and biodegrades in the sea naturally.
2 Bioplastic for Rigid food packaging	Food packaging Industry	Biosynthesised PHB and nanocellulose biodegradable polymer/ polymer blend sample films with equivalent properties to current PE packaging containers carried in collaboration with Logoplaste
3 3D printable Bioplastic Filaments	3D Printing manufacturers	Compatibilised biopolymer blends for 3D printing filaments,, carried out in collaboration with TCD, AIT and client companies will build on AIT's nationally funded EcoMixedPLas project.
4 Biosurfactants	Cosmetics, Pharmaceutical, Industrial & Household Cleaners, Agricultural reagents	Biosynthesised rhamnolipids with high functional biosurfactant properties.

Table 12- Expected bioplastic and biosurfactant products and corresponding user needs

The successful delivery of sample replacement bioplastic alternatives to the recalcitrant plastics within the food packaging and 3D printing markets along with rhamnolipid biosurfactants is aligned with the EU Commission's strategy on plastics and sustainable products. In particular, the BioICEP food packaging bioplastics are directly in line with EU's commitment to work towards the goal of ensuring that all plastic packaging is recyclable by 2030.<sup>9</sup>

#### IMPACT 4 - Description of a sustainable and environmentally friendly pilot system for the degradation of plastic mixtures

**BioICEP will deliver ONE sustainable and environmentally friendly 100L pilot plant for the integrated degradation of waste plastic mixtures and production of bioproducts.**

**#4**

A green energy powered integrated pilot scale system demonstrating the implementation the BioICEP technology will be set up at AVE's tech hall (Ghent, Belgium). This integrated pilot system will include a modular biocatalytic and microbial degradation bioreactor, a biomass separation unit, and a bioproduct fermentation bioreactor. A life cycle analysis will be performed to assess the pilot plant's and technology's sustainability and environmental friendliness.

The pilot plant will be operated to degrade at least 20% of mechano-biochemical pretreated mixed recalcitrant plastics and subsequent bioprocessing into PHB/rhamnolipid/nanocellulose bioproducts. A plan for a further more compactly integrated pilot plant will be developed to include incorporation of the mechano-biochemical pretreatment process for the potential of a all-in-one waste mixed plastic to bioproduct bioconversion system. Furthermore the potential for an ultra compact high efficient design, via the installment of a novel microbial platform with coupled all in one degradation-synthetic capabilities, will be assessed for recommendation. This design will be informed by life cycle analysis recommendations to ensure lowest environmental impact technology processes are selected.

An outline business plan illustrating the operational outputs and potential revenue streams for the pilot plant is presented in section 2.2.A.2.

<sup>22</sup> [https://www.marketsandmarkets.com/Market-Reports/biosurfactant-market-163644922.html?gclid=Cj0KCQIAvPjBRDPArisAIfZz0oHLWFBDf5DyNu\\_y\\_TFKBbn-jBW5yq4GrmB4tQXj3gtvH9dPPVPosoaAiusEALw\\_wcB](https://www.marketsandmarkets.com/Market-Reports/biosurfactant-market-163644922.html?gclid=Cj0KCQIAvPjBRDPArisAIfZz0oHLWFBDf5DyNu_y_TFKBbn-jBW5yq4GrmB4tQXj3gtvH9dPPVPosoaAiusEALw_wcB)

### International Cooperation

International cooperation and developments are essential within any mission to deliver a circular economy for plastics. International initiatives on litter, such as the UN Global Partnership on Marine Litter, G7 and G20 initiated action plans, the EU Commission's facilitated cross-industry dialogue on plastics management and series of international conferences, underline the growing global awareness of the nature of these challenges. The China-EU international engagement opportunity presented by this project provides an important opportunity to driving awareness and action beyond Europe's borders. AIT as the coordinator of the BioICEP consortium has an established presence in China with approximately 50 MOUs signed with Chinese universities and have two thousand students enrolled on AIT-certified courses in Chinese Universities. The Chinese consortium partners are from Beijing Institute of Technology, Shandong University, and Institute Of Microbiology Chinese Academy of Sciences and possess world-leading expertise in bioprocessing and bioproduct synthesis, plastics processing and treatment, and microbial and enzymatic bioengineering. AIT's EU-China engagement officer, is a Chinese national and has played a significant role in the establishment of the strong cross-consortium collaboration with the EU and Chinese participants.

The Chinese entities will contribute a number novel technologies to further strengthen BioICEP's capabilities on delivering the target of degradation of >20% of mixed plastics waste. These technologies include new *in situ* biosensors for expedited strain/enzyme screening and CRISPR-9cas technology for advanced directed evolution to bioengineering inflated plastics degradation efficiencies. These universities will also provide access to wild-type microorganisms which have evolved naturally in the presence of plastic waste and will be critical to new microorganism developments. This strong cross-continent collaboration will be essential to the achievement of the BioICEP technologies. The fact that plastic value chains are increasingly cross-border and cross continent will drive further international cooperation. Therefore the seeds sown by the BioICEP EU-China initiative will readily permeate internationally. Our first direct evidence for this is the very recent request by a Canadian company to enter into discussions with the BioICEP team. The vision, the revolutionary biotechnology, key information, and innovative bioproducts and pilot demonstration planned throughout the project will serve to communicate and **deliver the BioICEP message on an international stage** and motivate international cooperation.

Table 13 - International Cooperation

**BioICEP** will organise ONE international conference, TWO workshops and attend at least SIXTEEN international conferences and FOUR trade fairs to **enable cross-border and cross-continent stakeholder engagement**.

#5

**BioICEP** will enter into direct contact with at least **TWO HUNDRED international companies** by using the consortium's 600+ specialist company network to enable them to transition from petroleum based plastics.

#6

### Innovation capacity

The BioICEP technology holds significant innovation capacity for a range of distinct markets. These markets are driven by the increasing demand for sustainable products and by consumers and brands. Emerging markets arising from the continuous advancements and innovations of the bioplastics industry are primed for new bioproducts and materials with improved properties and new functionalities. High performance packaging and 3D printing filaments, as targeted by BioICEP, are among the highest growth segments.

Currently, bioplastics represent roughly one percent of the 335 million tonnes of plastic produced annually. But as demand is rising, and with more sophisticated biopolymers, more applications, and as new products are emerging, the market is continuously growing. According to the latest market data compiled by European Bioplastics in cooperation with the research institute, nova-Institute, global bioplastics production capacity is set to increase from around 2.11 million tonnes in 2018 to approximately 2.62 million tonnes in 2023.

The BioICEP consortium will aim at creating a spin-out company in 2021 will commercialise the project's expected innovative processes, products, and services. This will be in line with AIT's ambitious programme for the spin-out of applied research ventures from its Research Institutes and Technology Gateways Consortium. AIT's commitment to promoting a commercialisation and spin-out culture and creating spin-out companies that progress to become High Potential Start Ups is supported and funded by Ireland's national Technology Transfer Strengthening Initiative. Funding opportunities will be leveraged including the EU SME and InvestEU instruments to support the delivery of BioICEP innovative products to market. BioICEP products will have significant knock-on impacts enabling and facilitation further innovation within the bioplastic and bioproduct industry chain. A cyclical innovation process within consumer products will feed a demand for increased functionality and promote continuous enhanced bioproduct development.

Table 14 - Innovation capacity

**BioICEP** will create **ONE spin-out company** in 2021 to commercialise the project's expected innovative processes, products and services.

#7

## New market opportunities

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Market validation and the high value potential of BioIcep will be further assessed as part of the business plan development in WP8. To date, discussions with and letters of support (see Annex) from industry players outside the consortium strongly indicate the need for innovations in the plastics waste recycling and bioplastics production fields. BioIcep already has identified several companies interested in the technology including: Shabra in Ireland, Genecis in Canada, Esensa in Serbia and United polymers in Portugal. All have expressed high degrees of interest and support for the new technology, evidenced through several letters of interest, expressing a desire to assess the project outcomes with a view to discussing licencing options. Following the demonstration of the expected BioIcep results, all have indicated they would be willing to evaluate the technology for their own individual requirements with a view to negotiating investment and licencing options.

Table 15 - New market opportunities

**BioIcep will negotiate SIXTEEN evaluation licenses**, four of which will be with the consortium's SME partners, to validate the high value bioplastics market potential and **develop new market opportunities**.

#8

## Competitiveness and growth of companies

BioIcep will promote the creation of innovative technology-driven jobs serving to increase the income and business opportunities for stakeholders and actors within the waste management industry, plastic value chain, and bioproduct-based industries. Significant new employment opportunities will be generated in a number of distinct areas such as waste plastic pre-treatment and depolymerisation. Further innovation in bioprocessing-based manufacturing will be stimulated by BioIcep's development of a new high sustainable non-biomass dependent carbonaceous resource, which will be instrumental in enabling companies to overcome the current cost limitations of the biomass-based economy. According to [european-bioplastics.org](http://european-bioplastics.org),<sup>23</sup> the European bioplastics industry "could grow from 23,000 employees in 2013 to 300,000 high-skilled jobs in 2030". The association ascertains that "the bioplastics industry could provide new impulses for the development of rural areas in Europe by presenting new opportunities for the agricultural sector and consequently contribute to re-industrialisation and employment growth in Europe". The upgraded bioplastics and bioproduct developments offered by the BioIcep technologies will facilitate increased company competitiveness and generate commercial and employment growth within these markets. In addition opportunities for industries to become informed and enabled to transition from current petroleum plastics dependencies and adopt the new bioproducts and bioprocesses will also strongly benefit EU and global job creation.

The industrial partners within the consortium will benefit from a number of perspectives, including acquiring new cutting edge knowledge for product development and improvement. This will minimise the investment risk for the companies and lead to increased licencing opportunities and direct sales generation. Each of the consortium industry participants are operating in distinct but complementary areas key to the project objectives including: waste management, microbiome development for waste treatment, bioproduct production, and bioprocessing. For example, AVE intends to industrialise commercial bioreactors and specialised microbiomes for plastic degradation and depolymerisation, funnelling the outputs for bioproduct production. The knowledge acquired by participating in the BioIcep project and the opportunities to develop and licence in new IP will be instrumental in the business development of these companies. New employment opportunities will be created to support increased production, sales, administration, quality control, and R&D towards further exploitation of these innovations within the consortium companies.

Table 16 - Competitiveness and growth of companies

**BioIcep will provide a route to company cost savings of FIVE to FIFTEEN percent**, enable a **TEN fold job creation** increase potential and a **TWO fold increase potential in innovative products** to bioplastics manufacturing companies by demonstrating an alternative to biomass.

#9

<sup>23</sup> <https://www.european-bioplastics.org/faq-items/how-many-jobs-could-be-created-in-the-bioplastics-industry-until-2025/>

## Climate change and the environment

The contemporary bioeconomy, which is flagged to bring green and sustainable solutions for a circular economy, is dominated by the biomass-based economy. However progress is limited and over the past decade the amount of biomass used in the chemical and plastic industry in the EU has almost stagnated.<sup>1</sup> Strong limiting factors for biomass based products include the lower cost of petroleum based plastics, direct competition with food production and significant GHG emissions associated with the cultivation and processing of products from biomass. **In contrast**, new biotechnological processes using microorganisms and/or enzymes to convert carbonaceous resources, either biomass or depolymerised plastics into a broad range of different bioproducts are recognised for their high potential for reduced energy consumption and reduced GHG emissions.<sup>2</sup> The BioICEP next generation biotechnology platform is primed to accelerate the biomass bioeconomy and strongly contribute to a series of important sustainability targets and goals. BioICEP will contribute to 12 out of the 17 UN Sustainable Development Goals (SDGs), which contain targets to improve waste and resource management directly and contain the highest proportions of targets aiming to alter waste and resource flows in our economy.<sup>3,4</sup> BioICEP is designed to directly impact the global goals on: i) achievement of affordable and clean energy via very low overall energy consumption associated with the triple action depolymerisation and biosynthesis processes; ii) clean water, sanitation and life below water, by alleviating the streaming of microplastics into water systems; and iii) life on land, by the low carbon footprint biotransformation of enormous mixed plastic waste stockpiles into equivalent ubiquitous recalcitrant plastics replacements with biodegradable products. Additionally, the outputs of BioICEP will facilitate industries to respond to the EU call “to swiftly come forward with an ambitious and concrete set of voluntary commitments to back this strategy and its vision for 2030”.

Table 17 - Climate change and the environment

**BioICEP will give plastics value chain companies a solution to cut their GHG emissions by TEN to TWENTY FIVE percent and waste management companies a solution to cut their GHG emissions by THIRTY to EIGHTY percent.**

**#10**

## Other societal benefits

The size of the plastics and bioplastics production and recycling industry in EU and China are considerable. New innovative technologies, the retention and growth of high tech jobs within these sectors and the provision support in the momentous transition away from petroleum based plastics are needed. Selected output technologies from the BioICEP work, including defined high performing microbial consortia and enzyme cocktails for plastics degradation and bioproduction, will be licenced to plastic manufacturers and recycling industry players. In addition to the creation of new jobs and the retention of existing jobs, BioICEP innovative pipeline of bioproducts will benefit several large industry sectors spanning cosmetics and pharmaceuticals to 3D printing and additive manufacturing. In particular, the food production sector will benefit, both with respect to new biodegradable packaging and the alleviation of biomass cultivation. Most importantly, BioICEP will deliver key socio-economic benefits by the provision of a methodology to reduce plastic pollution, GHG emissions, and contribute to safeguarding the environmental health of our planet.

Table13 - Other societal benefits

**BioICEP will offer TEN short-term internship and secondment opportunities for researchers to maximise the technology transfer between the consortium partners and also to enhance dissemination & exploitation activities.**

**#11**

A key impact of the BioICEP project will be the demonstration of waste plastics as a low carbon footprint and high cost efficient non-biomass based rich carbonaceous resource for bioproduct development. The overall impact of the BioICEP value added products will deliver important contributions to:

- ☐ Demonstration of degraded waste plastics as a low carbon footprint cost effective alternative to biomass;
- ☐ Increasing plastic packaging waste recycling to meet 2030 targets;
- ☐ Facilitating producers in meeting new government and EC standards and responsibilities;
- ☐ Enabling amounts of municipal waste going to landfill to be reduced to less than 10% by 2035;
- ☐ Meeting new restrictions on incineration; and
- ☐ Facilitating Member States to meet EC environmental standards.

The suite of BioICEP technologies are designed to drive the global mission to transition to the sustainable management of plastics through the bioinnovation of a circular economy for plastics. This section describes in detail how BioICEP will achieve or surpass the expected impact goals outlined in the CE-BIOTEC-05-2019 call and positively contributes to 12 out of the 17 UN Sustainable Development Goals (SDGs), adopted by to 193 UN countries as highlighted in Figure 4 below. Moreover, the outputs of BioICEP will also support the priorities set by the EU Commission for an Energy Union for a modern, low-carbon, resource- and energy-efficient economy and the Paris Agreement.

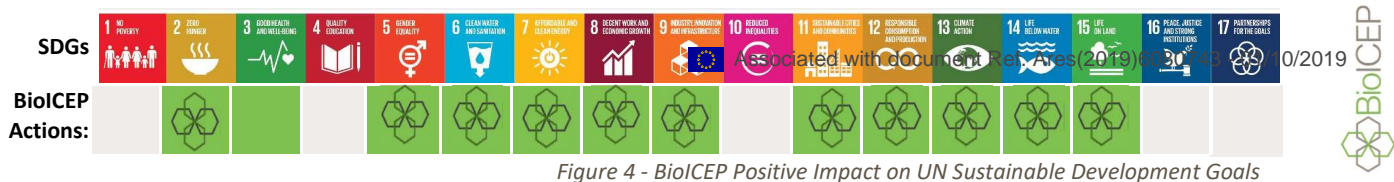


Figure 4 - BioICEP Positive Impact on UN Sustainable Development Goals

BioICEP will contribute to **TWELVE** out of the seventeen **UN Sustainable Development Goals**.

**#12**

### How the project will deliver each of the four specific output indicators listed in the impact section

The ESR Impact section states *“Expected impacts are clearly stated, by introducing estimated and quantified data, as mentioned in the call topic. Twelve key actions will be implemented to efficiently contribute to each of the expected impacts, with well described methodology and resources. Key indicators and metrics are presented, but how the project will deliver each of the four specific output indicators listed in the impact section of the topic description is not clearly demonstrated. For example the proposal targets most of the abundant plastic varieties independently and aims at degrading 20% of mixtures. However, the data of the fractions of plastics are not sufficiently explained. Goals for each type of plastic are given among them PVC and PS, which is extremely challenging, but the proposal fails to give details about how exactly PVC and PS shall be degraded.”*

New information has been added into proposal Table 11 (Expected degradation percentages for specific plastics within mixtures that are typically dispatched for landfill) in order to further explain the data fractions of the target plastics degradation. For each estimated percentage of each type of plastic, both recalcitrant and biodegradable, contained within a typical commercial plastic mixture, given in column 1, column in added which provided the expected percentage degradation using the BioICEP technology I each case. The degradation by weight following stage I +II and stage III of the BioICEP depolymerisations are listed in columns 3 and 4 of Table 11. These values have been compiled based on the considerable expertise of the consortium partners and our evaluation of the capacities BioICEP technologies. A further column 5 has been added which shows the expected percentage degradation of the individual original plastics and this information is plotted in the new figure P27 below.

Plastic	% Mixture (by weight)	Expected % degradation of Original plastic (by weight)	Indicator: % degraded post Depolymerisation I + II (by weight)	Indicator: % degraded post Depolymerisation I + II + III (by weight)	Indicator: % degraded of original individual plastic (by weight)
PE	29±3	10	2.9±0.5	3.7±0.5	12.76
PP	19±3	5	0.95±0.5	1.2±0.5	6.3
PVC	12±2	5	0.6±0.2	1.1±0.5	9.17
PS	8±2	20	1.6±0.5	2.5±0.5	31.25
PET	6±1	50	3±0.5	5.2±1	86.67
PU	7±1	30	2.1±0.5	3±1	42.86
PLA	1±0.5	70	0.7±0.2	1±0.3	100
Starch	1±0.5	100	1±0.3	1±0.3	100
Additives	17±3		NA	NA	NA
Total Mixed Plastics % (incl. additives etc.)	100		15.5±2.5	22.5±3	22.5±3
Total Recalcitrant Mixed Plastics % (excl. additives)	98		13.8±2	20.5±3	

Table 4 - Expected degradation percentages for specific plastics within mixtures that are typically dispatched for landfill or incineration



The ESR Impact section also states “The Goals for each type of plastic are given among them PVC and PS, which is extremely challenging, but the proposal fails to give details about how exactly PVC and PS shall be degraded.”

The consortium agrees with the ESR statement relating to difficulty in degrading PVC and PS due to the lack of hydrolysable functional groups. As such target degradation for PVC and PS is far lower than the levels expected for PU and PET recalcitrant plastics as highlighted in the graph in Figure P27. 9% of PVC and 31% of PS by weight will be degraded to using the BioICEP triple stage degradation approach. The first stage mechano green chemical techniques described in WP 2 will be used to reduce the molecular weight (MW) of the base polymer and increase making it amenable to biodegradation. In particular, the new proprietary sonic-green-chemical technology available at TCD is expected to strongly facilitate oxidation of the highly stable backbone carbon-carbon (C-C) bonds, provide functional groups including carbonyl or alcohol groups as well as increase hydrophilicity, enabling further depolymerisation. consisting of both bacterial and fungal species from BioICEP partners’ biobanks and literature strains reported for their ability to make recalcitrant plastics (especially ones with carbon-carbon backbones such as PVC and PS) more amenable to enzymatic depolymerisations. The subsequent biodegradation stages will include digestion using enzymes enhanced through a range of innovative techniques, including accelerated screening utilising novel fluorescent sensors coupled with directed evolution; and microbial consortia developed from best in class single microbial strains from BioICEP partners’ bacterial and fungal biobanks and from literature strains reported for their ability to make recalcitrant plastics (especially ones with carbon-carbon backbones such as PVC and PS).

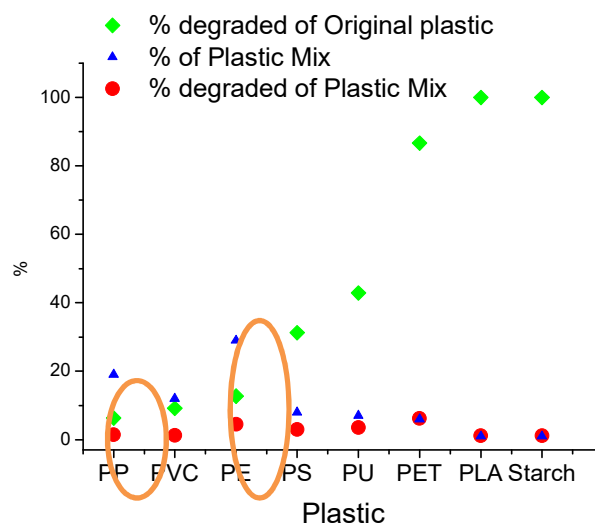


Figure. Mixed Plastic target percentages for (green – degradation of individual original plastic, blue, ie column 5 of Table 11 – individual plastic percentage of plastic mix ie column 1 of Table 11, red – degradation of total plastic mix including additives

9% of PVC and 31% of PS by weight will be degraded to using the BioICEP triple stage degradation approach. The first stage mechano green chemical techniques described in WP 2 will be used to reduce the molecular weight (MW) of the base polymer and increase making it amenable to biodegradation. In particular, the new proprietary sonic-green-chemical technology available at TCD is expected to strongly facilitate oxidation of the highly stable backbone carbon-carbon (C-C) bonds, provide functional groups including carbonyl or alcohol groups as well as increase hydrophilicity, enabling further depolymerisation. consisting of both bacterial and fungal species from BioICEP partners’ biobanks and literature strains reported for their ability to make recalcitrant plastics (especially ones with carbon-carbon backbones such as PVC and PS) more amenable to enzymatic depolymerisations. The subsequent biodegradation stages will include digestion using enzymes enhanced through a range of innovative techniques, including accelerated screening utilising novel fluorescent sensors coupled with directed evolution; and microbial consortia developed from best in class single microbial strains from BioICEP partners’ bacterial and fungal biobanks and from literature strains reported for their ability to make recalcitrant plastics (especially ones with carbon-carbon backbones such as PVC and PS). Sources of plastic waste from highly polluted global sites including China and Serbia, will be used to isolate new strains using conventional approaches, the iCHIP method and new accelerated screening methods. The selected microbes will be characterised by Chinese and European partners for their ability to degrade targeted plastics after pre-treatment, depolymerisation enzyme activities, providing an information

feedback loop to WP2, WP5, and WP6. The best strains from all screens will be used to identify and isolate novel enzymatic activities (WP3) and to create a defined consortium in WP5, which can breakdown mixed plastic. 'Designer strains' will be generated to boost plastic hydrolysing capacities based on microbial host platforms such as *Streptomyces lividans* and *Pichia pastoris*.

A schematic of the approach to novel enzymatic activity development and identification involving biocatalytic and enzymatic cocktail development for the mixed plastics degradation is given in Figure 2. Enzymes such as esterases, lipases, and cutinases are hydrolases will be deployed. The enzyme hydroquinone peroxidase, for example has been reported for its successful PS degradation PS. Approximately 100 enzymes will be selected amongst those reported in the literature for their ability to depolymerize each of the selected plastic materials. The enzyme hydroquinone peroxidase, for example has been reported for its successful PS degradation. Mutagenesis libraries will be screened using established high-throughput assays or the new biosensors of the degradation products developed by CAS. We will focus on the laboratory evolution of single genes, which we have identified as promising for the depolymerization of these plastic materials including PVC and PS. Site-directed mutagenesis has been performed, leading to improved activity of the selected enzymes.

This cumulative triple stage degradation technology, further details for which are provided in WP 2, 3, 4 and 5 will be used to achieve impact 1 and 2 of the four specific output indicators listed in the impact section. The details for the achievement of impact 3 and 4 of the four specific output indicators listed in the impact section are provided in WP 6 and 7 respectively.

**In order to achieve impact 3**, different microbial consortia and enzyme cocktails, developed and demonstrated in WP3, W4 and WP5 for their ability to synthesize one or more of the target products will be used to develop and optimize bioprocesses for their high yield production. The selected strains (up to 4) will be cultivated under optimized operational conditions, including monomer concentration, medium composition and feeding strategies, aiming at defining optimal cultivation conditions in order to get high productivities in a reproducible way. Metabolic models will be used to optimize the feeding strategies of key compounds including digested plastic waste compounds Fermentations and will be analysed and Principal component analysis (PCA) models will be developed with the results used for the development and validation of partial least squares (PLS) models for the in-line prediction of biomass, PHB, PHA, nanocellulose, and rhamnolipids content. The produced products will be recovered from the broth and characterized to evaluate their characteristics that will be evaluated as criteria for strain selection. The results of product chemical, mechanical, and thermal testing will feedback allowing the bioprocess to be refined and optimised for the generation of high quality bioproducts with properties appropriate to applications in market segments such as the food and pharmaceutical industryProcess. Validation is planned in a laboratory scale 10 L reactor, allowing definition of cultivation protocols for further scaling-up and development of guidelines for process design for the implementation of the processes at pilot scale in collaboration with AVE in WP7. Demonstration of pilot production of PHB and nanocellulose for thin biopolymer film production for applications such as food packaging will be carried out using compounding, hotmelt extrusion/extrusion and blow molding with the processing parameters optimised for thin film production. The materials performance of the thin films generated will be compared with the equivalent recalcitrant plastics currently in use in the relevant market applications in order to adjust process parameters and understand material behavior based on the input of WP 6. Prototype mould and parts design and construction will be considered in order to adapt to the material requirements. Process parameters and suitability of the material rheology will be provided and the information fed back to enable biosynthesis improvements. Sample packing will be produced and characterised and customized into final geometries.

**Impact 4 of the four specific output indicators** listed in the impact section will be achieved by the construction of a process controlling and automatized pilot plant. This integrated pilot system will include a modular biocatalytic and microbial pretreated plastics degradation bioreactor, biomass separation, and bioproduct fermentation operated in accordance with the parameters developed in WPs 2-6. The reactor setup will consist of three operation units: (i) biocatalytic degradation of the pretreated plastics using enzymatic cocktails followed by consortium of strains, (ii) separation of the biomass and residual plastic components from the nutrient-rich fluid (using disk centrifugation), and (iii) microbial PHB/rhamnolipid/nanocellulose production using the nutrient-rich effluent stream of the first reactor as influent. Inocula will be used to start the pilot-scale reactor using at least 1-10% v/v inoculum, consisting of



viable biomass in the range of 2-3 g VSS/L. The influent stream will consist of pre-treated plastics, optimized during previous WPs and produced in sufficient amounts to enable operation of the pilot with predetermined active volume and for a predetermined duration. The fully characterised pre-treated plastics will be supplied by consortium partners and micronutrients and nitrogen and phosphorus sources will be added as specified. The microbial community composition can be monitored by amplicon sequencing. The degradation of pre-treated plastic will be measured using respirometry methods. Extraction and purification of PHB, nanocellulose, and rhamnolipids will be carried out using environmentally friendly protocols developed in WP6 ready for characterization and processing testing. Extensive chemical, mechanical, thermal and aging characterization and analysis will be carried out. The modular operation will allow the regulation of biocatalytic and microbial pretreated plastics degradation processes in accordance with the parameters developed in WP 3 and 5. These will be selected in order to provide the most suitable depolymerised mixed plastic feedstock for the fermentation of the required bioproduct according to the protocols developed in WP6. The generation of PHB/rhamnolipid/nanocellulose bioproducts will be optimised and fully characterised to ensure their potential for end of use applications.

#### 2.1.4. Barriers and obstacles to expected impacts achievement

The BioICEP consortium is acutely aware of and working to overcome the barriers inhibiting the plastic waste recycling market, many of which are interlinked and reinforce one another.

The technical barriers, which need to be overcome to enable the successful delivery of the BioICEP solution, requires investment in infrastructure and innovation. Such investments are held back by the uncertainty over the supply of material, the quality of the recycled material, its competitiveness compared to virgin plastics and thus the size of the market. The volatile price of virgin plastic also presents a significant problem for the plastic recycling market, but this cannot be directly controlled. Extracting the plastic waste from municipal waste, particularly plastic packaging, is a key challenge and one that will require investments in infrastructure, but also requires innovation in sorting and treatment technologies.

The overwhelming size of the petroleum based plastic market size relative to the emerging bioplastics market size is another substantial barrier, as seen in Figure 5. Bioplastics currently account for 1 per cent of the approximately 320 million tonnes of plastic produced annually, and bioplastics manufacturers have to compete with conventional plastic production, a remarkably inexpensive process scaled over the past 60 years by the oil industry. However, the size and continued growth of the fossil-based plastic production representing the enormous potential of the BioICEP industry concept.

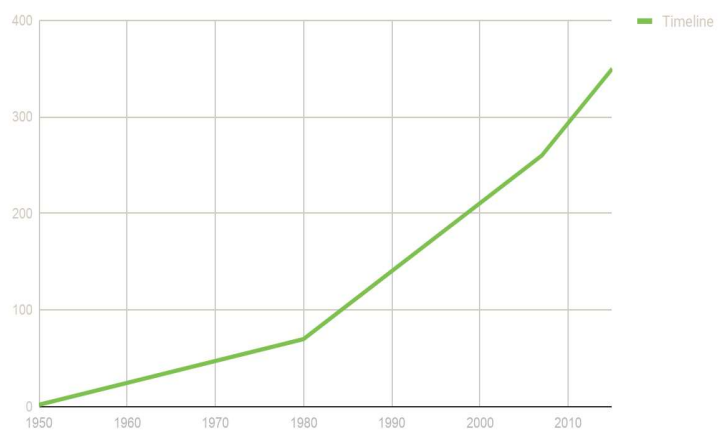


Figure 5 - Plastics Production from 1950 to 2017

Source: "Bio-based Building Blocks and Polymers – Global Capacities, Production and Trends 2018 – 2023", Nova-Institute

Two reports have been initially conducted and will be further refined towards understanding the barriers and obstacles to BioICEP impacts' achievement: a) a **Porter Five Forces analysis** to examine the barriers expected to be found during market penetration; and b) a **S.W.O.T. analysis** to analyse the opportunities and risks for the adoption of the BioICEP technology. The results of these reports can be found in the Tables below.

#### Porter Five Forces analysis:

##### Competitive Rivalry:

- Bioplastics is a very promising and constantly growing market. New companies are emerging and are implementing new ways to address the demand. The current bioplastics market growth rate is around 20% per year.<sup>24</sup> Furthermore, bio-sourcing and biodegradability have recently emerged as major market drivers especially in the packaging sector. With BioICEP, new and innovative technologies for plastics biodegradation and development of new bioplastics with similar properties to currently available plastics will be developed. One of the main challenges will be to compete with the high

<sup>24</sup> [www.european-bioplastics.org/market](http://www.european-bioplastics.org/market)

level of research emerging in this domain. However, while threats from research performing organisations and SMEs could emerge, a lesser threat exists from large plastic manufacturing companies, due to the fact that, while they are quite advanced in the domain of bioplastics, they have not yet found sustainable solutions on recycling the products of their current production line. BioICEP provides a new pre-treatment process which goes a step beyond the current market solutions.

#### Threat Of New Entrants:

- The 2018 European bioeconomy market is worth 2 trillion euros in annual turnover and accounts for 22 million jobs in the EU, which is 9% of EU's workforce. The number of companies working directly on bio-waste treatment is growing, but solutions still need to be found, which is why more companies are expected to enter the market in the forthcoming years. The BioICEP project takes into this situation account and goes steps beyond the state of art and the expected sector development in the following years. The European bioplastic economy has the potential to be the world's most extensive network of bio-based companies which can actively participate, grow, and develop new products and processes. BioICEP will carefully consider Intellectual Property Rights issues to ensure an effective protection and further exploitation of results.

#### Threat Of Substitute Products:

- Tools, techniques, and processes leading to a better approach of plastics treatment can be considered as potential threats of substitute products or improvements on the solutions given by BioICEP. Highly innovative solutions to target the demand of the bioplastics and plastics recycling market in Europe will be provided by BioICEP. While no substantial threat of substitute products exists as of now, BioICEP will closely watch new plastics treatment technologies being developed during the lifetime of the project and beyond.

#### Bargaining Power Of Suppliers:

- The BioICEP project focuses on innovative technologies developed by research centres and engineering companies. Feedstock suppliers are plastics, waste management / treatment and micro-organism producer companies, which are widely spread across Europe and have already a consolidated market. BioICEP will provide compositions of different microorganism cultures that will have the potential to treat almost all types of plastics without any sorting. Marginal difficulties are expected to be encountered in finding suppliers, taking into account the maturity of the market and the large amount of suppliers.

#### Bargaining Power Of Buyers:

- The potential buyers of the BioICEP products are going to be progressively in touch with these innovative technologies and demanding better quality in bioplastic materials and added value products. The expectations of market growth and continuous development are quite optimistic, which leads to better market penetration. High bio-based added value products are constantly increasing their acceptance in society and this trend is expected to continue. In addition, strong efforts will be made during the BioICEP project to probe the technical and economic feasibility of the final products, increasing the confidence of end users in the usability of bioplastics and recycling of plastics.

Table 19 - Porter Five Forces analysis examining the expected barriers to market penetration

BioICEP S.W.O.T. Analysis:	
Strengths	Weaknesses
<ul style="list-style-type: none"> <li>□ Decrease waste</li> <li>□ Low cost treatment process</li> <li>□ Environmentally friendly process</li> <li>□ Solution to decrease plastic waste</li> <li>□ Motivated and adaptive industrial partners</li> <li>□ Supply chain management</li> <li>□ Proteins &amp; BACs fully based on bio-based feedstock</li> <li>□ New bio-based value chains</li> <li>□ Cross sectoral market approach (fisheries, pharmaceuticals, food, feed)</li> </ul>	<ul style="list-style-type: none"> <li>□ Increased population pressure/ lifestyle demands which might prohibited the public acceptance of bioplastics</li> <li>□ Consumer acceptance</li> <li>□ Strict regulation/legislation framework</li> <li>□ Consumer acceptance and new to the market</li> <li>□ End users are relatively reluctant to accept new technologies</li> <li>□ Investment in new business models and logistics are required</li> <li>□ Pricing policies and valorisation paths</li> </ul>

<ul style="list-style-type: none"> <li>Advanced product functionality (high protein, high BACs, appropriate purity)</li> <li>Strong scientific and technological knowledge in the fields</li> </ul>	<ul style="list-style-type: none"> <li>Difficult to establish new branding codes and products in the market</li> </ul>
Opportunities	Threats
<ul style="list-style-type: none"> <li>Several EU countries have adopted the fee rule for citizens using plastic in products. The increased cost of plastic products is an opportunity for new products to get out in the market</li> <li>Opportunity for EU companies to adopt a new way to produce bioplastics in an economic way</li> <li>Creation of new jobs in these sectors</li> <li>Development of green economy strategy</li> <li>Provide services for new SMEs and product development</li> <li>Extremely high market potential</li> <li>Scope of product line expansion</li> <li>The developed products will comply to the new EU regulation for green and sustainable production</li> <li>Provide desired properties to end products from residual side streams and low energy technologies</li> <li>Diversification through services</li> <li>Volume of waste produced</li> <li>EU/China legislative changes</li> </ul>	<ul style="list-style-type: none"> <li>Competing technologies</li> <li>Reusable materials which can replace plastic all together such as glass. however they consume huge energy to recycle</li> <li>Competitors making tougher to retain the point of difference</li> <li>Need of strong IPR policy</li> <li>Threat of new entrants in European market</li> <li>Competition between similar companies and services</li> <li>Regulatory approval processes for food contact applications</li> <li>Close cooperation between parties in the value chain</li> <li>Target markets to re-learn “value selling”</li> </ul>

Table 20 - S.W.O.T. analysis of the opportunities and risks to the BioICEP technology adoption

## 2.2. Measures to Maximise Impact

### 2.2.A. Dissemination and exploitation of results

The BioICEP consortium members have collated a targeted, transparent and efficient dissemination, exploitation and technology/knowledge transfer strategy to maximise and promote the outcomes of the project.

Priority dissemination, exploitation and technology/knowledge transfer measures were identified by BioICEP consortium members to facilitate outreach and uptake of products, processes, and results from the BioICEP project following completion of a consultation with all members of the consortium. BioICEP partners, including SME partners with insight into multiple sectors, have highlighted priority dissemination activities warranting attention to bridge the void between research and innovation thus fast-tracking the progression of innovative BioICEP products to market.

Dissemination & exploitation, as well as communication and outreach activities, will inform and engage relevant BioICEP stakeholders, including researchers, industries, policy makers, and society. To ensure efficient implementation of the dissemination, exploitation and technology/knowledge transfer strategy all BioICEP consortium members will receive training on key Horizon 2020 priority areas related to data management and dissemination activities. Training will be provided during the kick-off meeting and will be

The successful exploitation of BioICEP projects' results will be achieved by:

- validating the technology developed;
- promoting market awareness; and
- understanding, supporting and involving a range of stakeholders regarding the technologies' developments, potential and subsequent application (products, services, processes, etc.).

The partners will analyse and validate the primary and secondary market potential and structure a go-to-market strategy accordingly as part of the post-project activities. Cooperation with regulatory bodies and third party sales and distribution licensees in the production markets will be implemented. This process has already begun through the concept development and pre-project market validation work carried out by the partners within their existing customer bases and will be further updated during the project. The final PDER, which will be released in Month 48, will be drafted according to Intellectual Property Rights (IPR) restrictions and information resulting from:

- The knowledge in the production of microorganism communities for plastics bio-degradation;
- Evaluation of possible industrial outcome for the research results obtained during this project;
- Estimation of the economic impact of the products and technologies developed in the project;
- Observation of market trends and positioning of project results;
- Market analysis (actual market needs and size in Europe); and
- Standardisation and regulatory aspects.

Given the broad range of applications of BioICEP's results in society and of the project's products & processes, consortium members have highlighted **key priority dissemination objectives** to maximise the impact of BioICEP on society. Dissemination, exploitation and technology/knowledge transfer activities will include:

- Engaging at least **200** leading local, European and global companies, industry experts, policy makers and research groups, utilising BioICEP consortium **600+** members networks, dissemination boards and strategic boards;
- Presenting at **16+** conferences and hosting **1** international conference and **2** stakeholder workshops;
- Organising **50+** site visits, attending **20+** seminars, **4** trade shows and exhibitions;
- Disseminating BioICEP outcomes through **1** dedicated website and **5** social media platforms (Twitter, LinkedIn, Facebook, a YouTube channel and a blog) and **60+** publications (journal articles, newsletters, brochures and flyers);

#### 2.2.A.1. Draft Plan for the Dissemination and Exploitation of Results (PDER)

The project coordinator as chair of the **Dissemination and Exploitation Board (DEB)** – with the contribution of the whole consortium – will be responsible for the development of a **rolling Plan for the Dissemination and Exploitation of Results (PDER)**, including an operational protocol which will consider both internal and external exploitation factors to consider for the future commercialisation phase of the results as well as actions needed to bring project results to the market.

A detailed implementation plan with a timeline will be developed for all activities at the start of and during WP8, outlining key responsibilities and contribution from all relevant partners to the dissemination and exploitation (DE) activities. WP8 leader will act as DE manager and will carry out the coordination of all individual DE capacities of the partners, in order to achieve a bigger impact.

Below is a sketch depicting BioICEP's Dissemination and Exploitation Process Map:

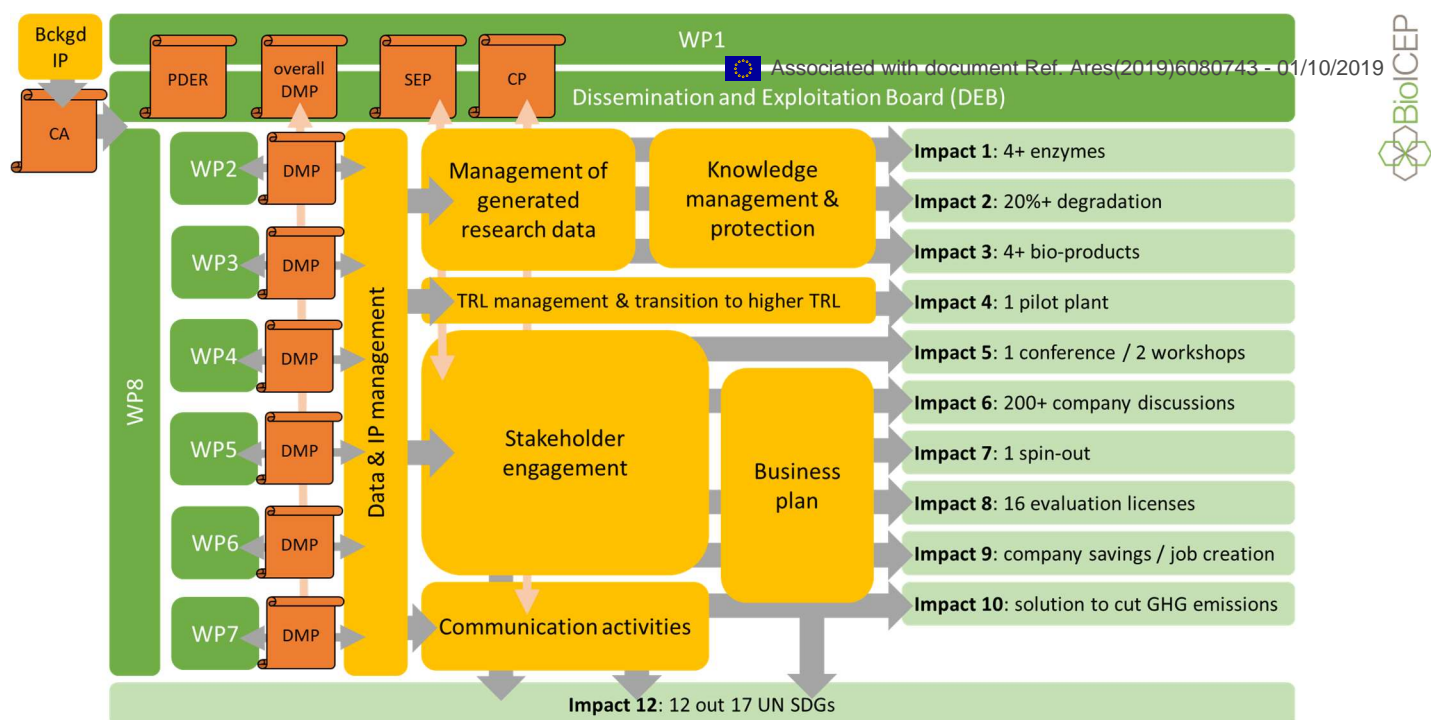


Figure 6. BioICEP's Dissemination and Exploitation Process Map

A short description of:

- an outline **Strategy for Stakeholder Engagement** (Dissemination/Exploitation);
- **Intellectual Property, Data & TRL management considerations** (Exploitation);
- a **Draft Business plan** (Exploitation);
- an outline **Strategy for generated research data management** (Dissemination/Exploitation);
- an outline **Strategy for knowledge management and protection** (Exploitation);
- the **planned Communication activities** (Dissemination) and their relative **Key Performance Indicators** (KPIs);

which will be developed as part of the PDER so as to achieve all of the targeted project's impacts is outlined below.

#### Strategy for Stakeholder Engagement (Dissemination/Exploitation):

The BioICEP consortium will draw on its extensive network of industry contacts and on its industry knowledge to reach the maximum audience so as to achieve the expected impacts of the work programme as well as the numerous other impacts (e.g. societal and economical) highlighted above in Section 2.1.

A Stakeholder Engagement Plan (SEP) will be developed as part of WP8/Task 8.1/D8.2. Stakeholder mapping will help understand each stakeholder according to parameters such as influence/power capacity, network capacity and interest level. Stakeholder engagement activities will be identified to identify engagement strategies according to each group and to stakeholders' mapping power/interest charts. Finally a stakeholder engagement plan matrix will show who BioICEP stakeholders are, how and when they will be reached.

A draft stakeholder engagement table, outlining the target groups, key project results, expected impact, and means of dissemination, is given below.

Target Group	Key Project Results (deliverables, technologies and/or products)	Expected Impact	Means
Industries using plastic as raw material	Compatible polymer blends formulations using pre-treated and microbial degraded plastic waste as a compatibiliser (WP2)	Inform 100 companies for the benefits of using bioplastics  Increase the market of bioplastics by increasing the clients	Participation of consortium members in trade fairs, events and developing an




Target Group	Key Project Results (deliverables, technologies and/or products)	Expected Impact  Associated with document Ref. Ares(2019)6080743 - 01/10/2019	Means
			electronic campaign
<b>Industries</b> culturing microorganisms in Europe and China	Microbial strains for plastic degradation (WP4&5)  Synthetic communities and or enzyme cocktails (WP5)	Inform 50 companies for the use of microorganisms in plastic recycling and bioplastic production  Increase the suppliers of microorganisms for the recycling process	Participation in trade fair, events and by following an electronic campaign in Europe and China
<b>Industries in waste treatment</b>	Novel pre-treatment processes to improve the accessibility of plastic waste for microbial degradation (WP2)	Inform 50 companies for the innovative recycling process of BioICEP  Negotiate licenses of BioICEP technology to interested parties	Participation to trade fairs, events and by following an electronic campaign
<b>European and Chinese policy makers</b>		Sound communication towards policy-makers to increase the influence of the project and its results in policymaking in Europe and China	Participation in policy making events and clusters

Table 21 - A draft stakeholder engagement plan

BioICEP will engage with and relay research outcomes to leading European, Chinese and global companies, end users, policy makers and industry experts/research groups, examples of which are given in the Table 22 below.

Examples of stakeholders:			
European, Chinese and global manufacturing companies	End users	Policy makers	Industry experts/Research groups
Agrana Stärke, API, BASF, BIO-FED, BIOTEC, CARBIOLICE, Danimer Scientific, DuPont, FKUR Kunststoff, Futamura, Indochine Bio Plastiques, Jinhui Zhaolong High Tech., Kaneka Corporation, Mitsubishi Chemical Europe, NatureWorks, Novamont, Perstorp, Succinity, Sukano, Synvina, Taghleef Industries S.p.A., TIPA Corp, Xinjiang Blue Ridge Tunhe Polyester, Zhejiang Hisun Biomaterials, local waste management companies	General public, Media, Industrial end users: e.g. Danone, Ferrero, Lavazza	European Union, Local and national authorities	European bioplastics Nova-institut, C.A.R.M.E.N., COBRO, Cofresco, Frischhalteprodukte, DIN CERTCO, Fraunhofer ISC, IFA Tulln, IfBB, Institut für Kunststofftechnik ISCC, Organic Waste Systems, Packbridge Roundtable on Sustainable Biomaterials, TÜV

Table 22 - Stakeholder examples

BioICEP will host one conference and two workshops aimed at the scientific community, industry leaders and the general public. Outreach events at the end of year 2, 3 and 4 will be hosted at BioICEP's consortium facilities (industry and academic) and will showcase the applications of and potential for BioICEP specific products and processes. Attendance at and hosting conferences will also serve to enhance presentation skills of consortium members, to increase awareness and to solicit feedback so as to maximise the impact of BioICEP products. New and existing industry contacts will follow up on industry/academia workshops to discuss and share novel processing techniques pre-competitively prior to publication/dissemination of these methods.

Examples of conferences/exhibitions that will be targeted are given below:

- International Conference on Bio-based Materials;

- European Bioplastics Conference;
- Annual Circular Economy Stakeholder Conference;  Associated with document Ref. Ares(2019)6080743 - 01/10/2019
- International Conference on Waste Management and Technology;
- World Conference on Waste Management; and
- International Conference on Technologies & Business Models for Circular Economy.

### Intellectual Property management (Exploitation):

Intellectual Property Rights (IPR) take into account that the respective Articles 23a-31 in the Grant Agreement must be respected. The Dissemination and Exploitation (DE) manager, the Project Manager (PM) and the Project Coordinator (PC) will coordinate and collate **background intellectual property (IP)** in the **Consortium Agreement** which will be circulated to and signed by all partners. Background IP refers to know how and expertise brought by each partner to the BioICEP consortium. **Access to Foreground IP** and veto rights to IP created during the BioICEP project will be clearly assigned to individual or multiple partners in the Consortium Agreement based on partner specialty and work. **Joint ownership of IP** will have clear guidelines in the Consortium Agreement including the allocation and terms of exercising ownership of joint foreground IP. The DE manager, the PM and the PC in conjunction with the project IP specialist partner will be responsible for resolving IP issues and protecting IP prior to and during the project including patent applications. **Additionally, the Consortium Agreement will provide clear guidance in relation to access to patents, licencing agreements, competitive activity and implementation commencement, and completion dates.** The option of providing the consortium industrial partners non-exclusive royalty-free licenses to evaluate intellectual property generated will be established within the Consortium Agreement. Intellectual property workshops and training seminars will be provided to address IP awareness and management, culturing processes, biodiversity and sharing implications on commercialisation, data management life cycle, and maritime law. These seminars will be available to BioICEP partners, industry contacts, and potential collaborators as appropriate.

Table 23 Intellectual Property Management

### Data management (Dissemination/Exploitation):

Data management life cycle, communication, and verification of all data sets is central to the remit of Horizon 2020. Therefore **BioICEP** will develop and implement **Data Management Plans (DMPs)** from templates provided by the EC and in accordance with “Guidelines on Data management in Horizon 2020” for all research activities. DMP templates will be included in the Project Handbook. DMPs will serve to identify, manage, curate, and preserve data which will be made freely accessible for verification or re-use by **BioICEP** consortium members. Therefore, BioICEP DMPs will increase the efficiency of its research by avoiding duplication of efforts in addition to providing access to data outside the consortium facilitating potential applications in multiple sectors. DMPs will facilitate the protection and submission of invention disclosure forms throughout the project. The Dissemination and Exploitation Board (DEB) will draft and circulate template DMPs for each work package and anticipated research outcomes.

In accordance with Europe’s 2020 strategy aimed at developing a smart, sustainable, and inclusive economy, BioICEP’s non-confidential data will be readily available to the public. Facilitating access to BioICEP data will be conducted in accordance with Horizon 2020’s “Open Research Data” initiative and Plan S developed to maximise access to and exploitation activities in research. BioICEP research outcomes will be openly available through ease of discovery, accessibility, intelligibility, identifiability, and usability. Thereby BioICEP data will be assessable and conducted in accordance with quality standards. Data Management Plans will be constructed to address self-archiving, open access publishing, and open access to research data. Access criteria will be addressed in the terms and conditions of the BioICEP Grant Agreement, which will be reviewed and signed by all partners prior to the project commencement, thereby facilitating effective and transparent dissemination and exploitation of BioICEP results among the consortium and society. Given the pan-European nature of the BioICEP consortium, the **DMP** will facilitate interoperable access and use of data between researchers, organisations and countries by standardising data management criteria to facilitate the combination and analysis of numerous data sets referred to as free or “**Gold Open Access**”. The preservation of data beyond the lifetime of the BioICEP project will ensure that the data generated is usable by third parties for wider sector applications beyond its original purpose. However, a situation may arise where access to BioICEP data will be curtailed or restricted, in this circumstance a clear description, rationale, justification, and embargo periods will be outlined in the Consortium Agreement and the Data Management Plan. Restrictions in data sharing may be due to IP issues or commercial sensitivity will be referred to as “**Green Open Access**”. BioICEP will adopt and implement Horizon 2020’s Open Research Data Pilot, insofar as possible whereby access to specific parts of BioICEP’s research data will be curtailed. Premature or immediate access to sensitive data generated within BioICEP could jeopardise the objectives of the project, IP, confidentiality issues, and successful completion of the project.

Table 24 – Data Management



## TRL management and transition to higher TRL (Exploitation):

The DE manager and the PC will play a crucial role at the interface between search and application. Equipped with a dissemination and exploitation plan, in addition to DMPs, the DE manager and PC will maximise the impact and advertisement of BioICEP results through data release or protection. The DE manager and PC will determine the optimal outreach avenues via the general public, scientific community, industry, various societies, and end-users at the end of the BioICEP project. It is envisaged that towards the end of the project, **BioICEP technology will reach TRL5 level**. Therefore dissemination and exploitation activities will focus on TRL5 in an effort to maximise impact and uptake, thus promoting the possibility of attracting additional funding (post project) to develop the technology to higher TRL levels. Armed with the combined expertise of all BioICEP partners and their proven leadership characteristics in previous EU projects, it is expected that BioICEP will deliver an innovative technology with potential pathways to products with pan-sectoral applications.

Table 25 - TRL management and transition to higher TRL

### 2.2.A.2. Draft Business plan (Exploitation)

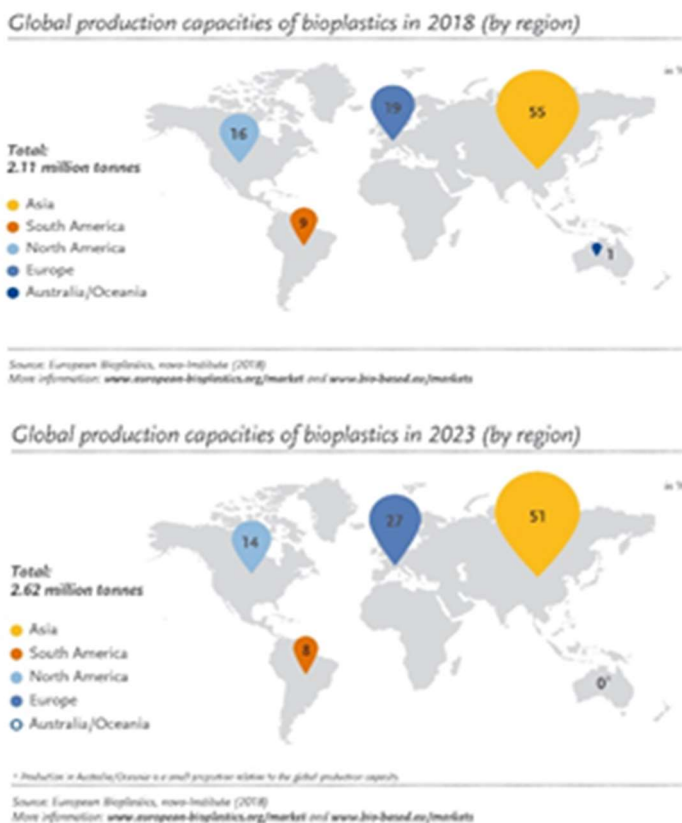
#### Geographical coverage and size of the target markets

Current market value of biodegradable plastics exceeds USD1.1 billion in 2018 and could reach USD1.7 billion by 2023 according to a report by IHS Markit, which given the production capacity of 912,000 tons (source: nova institute 2018, see below), leads to an estimated current **value of biodegradable plastics in the order of USD1.2k per ton**.

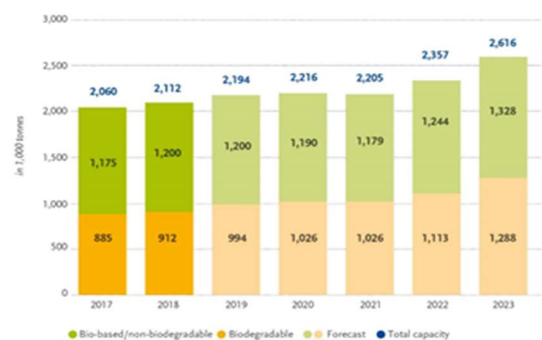
Currently, bioplastics represent roughly one percent of the 335 million tonnes of plastic produced annually. But as demand is rising, and with more sophisticated biopolymers, applications, and products emerging, the bioplastics market is continuously growing.

According to the latest market data compiled by European Bioplastics in cooperation with the research institute nova-Institute, global bioplastics production capacity is set to increase from around 2.11 million tonnes in 2018 to approximately 2.62 million tonnes in 2023.

Figure 8: Global production capacities of bioplastics by region



Global production capacities of bioplastics



Source: European Bioplastics, nova-Institute (2018)  
More information: [www.european-bioplastics.org/market](http://www.european-bioplastics.org/market) and [www.bio-based.eu/market](http://www.bio-based.eu/market)

Figure 7: Global production of bioplastics

Western Europe, with the world's most strict use regulations for single-use plastics, holds 55 per cent of the global market value in 2018 for biodegradable polymers, and it is growing. Europe is a major hub for the entire bioplastics industry. It ranks highest in the field of research and development and is the industry's largest market worldwide.

With a view to the actual production of bioplastics and regional capacity development, Asia is a major production hub. In 2018, 55 percent of bioplastics were produced in Asia. Around one fifth of the global bioplastics production capacity is located in Europe and this share is predicted to grow to up to 27 percent by 2023. The expected growth until 2023 will be supported by recently adopted policies in several European Member States, such as Italy and France.

BioICEP's main competitors are existing PHA/PHB producers. A list of in PHA/PHB producers is given in Annex E. They typically synthesise PHA/PHB from expensive refined carbon sources that often compete with the human food chain.

Other competitors include multinationals such as BASF (Germany), Total (FR), Solvay (Belgium), Dupont (USA) who are closely watching and developing novel bioplastic technologies and start-up companies such as Genecis (Canada), Venvirotech (Spain), Hafigate Hydal (Czech Republic) who are developing fermentation-based technologies to manufacture bioplastics from food waste.

The novelty of the BioICEP development will deliver the following benefits for global consumers and the plastics supply chain and recycling industries:

- ▣ Redirection of waste plastics for the production of high market need bioproducts;
- ▣ Sustainable bioplastics for non-disruptive replacement of petroleum plastics;
- ▣ Remove the need for the development of advanced plastic recycling facilities and collection logistics;
- ▣ Reduce recycling energy costs and carbon footprint;
- ▣ Overcome the depreciation and loss of value of current recycled plastics; and
- ▣ Pave the way for environmental clean-up and future sustainability.

A comparison to the best-in-class established and emerging bioplastic production companies, given in Table 26 below, highlights the unique capacity of **BioICEP** to provide an innovative solution to the global plastics challenges and underlines its potential to gain a world-leading technology position.

	Biodegradable, biocompatible & versatile	Not competing with food cultivation & supply	Lower carbon footprint production	Low energy production	Higher performing products than current bioplastics
<b>BioICEP</b>	High	High	High	High	High
<b>Emerging companies</b>	High	Medium	Medium	Medium	Medium
<b>Established competitors</b>	High	Low	Low	Low	Low

Table 26 - The BioICEP unique offering (compared to bioplastic production companies)

### Draft Business Model

During the project, the consortium will consider different business model options including potential markets, customers, distribution channels, etc. More specific and detailed information obtained during the work will be used to steer the final design, incorporating low environmental impact options and ensure the output incorporates the best compromise/s to fulfil the users' expectations. Moreover, the information gained will provide the basis to prepare proposals for the further **development of the technology in the post-project environment, further increasing the technology readiness level** and promote entry to the market.

The approach will be based both in direct gathering of information/experience and desk research of open literature, past European Commission research projects, and other research project where members of the consortium are participating. The foreseen business approach will be based on the work mainly in two aspects: the R&D of the whole product (improvements of designs, technical adaptations, etc.) and its commercialization (IPR issues, sales, licensing, etc.). Different business models will be developed from the basis of possible spin-offs, depending on different aspects, like IPR protection laws of different countries constructed and provided by suppliers (e.g. members of the consortium).

An preliminary Business Model Canvas (BMC) illustrating the operational outputs and potential revenue streams for the pilot plant is presented below. BioICEP's BMC is based on working directly with waste-management companies and elaborating waste management services and is illustrated in the business model canvas below:

Key Partners:	Key Activities:	Value Proposition:	Channels:	Segments:
Consortium partners as co-developers Consortium partners as producers	New product development New process development	Not competing with food supply. Lower carbon footprint production	Waste management marketing Direct contact with waste management companies	<i>Partners:</i> Waste management for technology licenses

Key Partners:	Key Activities:	Value Proposition:	Channels:	Segments:
Waste management companies as customers / license holders	<b>Key Resources:</b> IPR Internal knowledge Human resources & expertise	Low energy production Higher performing products that current PHA based plastics	<b>Relationships</b> To end-users and recycling product manufacturing companies, via waste management companies To waste management companies via internal marketing team	End-users Recycling product manufacturing companies General public
Revenue Streams		Costs		
<b>Value-based</b>		<b>Value-based</b>		
<ul style="list-style-type: none"> <li>Waste handling upfront payments</li> <li>Product manufacturing companies payments</li> <li>Licensing Payments</li> </ul>		<ul style="list-style-type: none"> <li>Overheads/fixed costs</li> <li>Cost of process / product development</li> <li>Legal costs for technology licensing agreements</li> </ul>		

Table 27 - The BioICEP Business Model Canvas

BioICEP's cost and revenue structures are centred on profits via generating value for partners and customers, rather than minimising costs for themselves. Reasons for selection of this structure include ensuring that adequate protection for the company and its innovations are accounted for in every agreement, and ensuring protection of the partner's interests.

BioICEP business plan's financial projections, as currently envisaged, are based on the following assumptions:

- an estimated market penetration rate of 0.1% at Y-1, rising to 10% at Y5
- an estimated market CAGR of 15%;
- an estimated plastic waste conversion rate of 20 per cent;
- an estimated charge/cost of plastic waste (goods in) of €100 per ton;
- an estimated value of end products (goods out) of €1,000 per ton; and
- an estimated cost of production, primarily based on the cost of enzymes, of €50 per ton.

Table 28 – Financial Projections Assumptions

#### Sales Forecast:

Year	Y-4	Y-3	Y-2	Y-1	Y1	Y2	Y3	Y4	Y5
	2021	2023	2024	2025	2026	2027	2028	2029	2030
TRL	TRL3	TRL4	TRL5	TRL7	TRL9	commercialisation			
Market size	€1.5b	€1.7b	€1.9b	€2.2b	€2.6b	€2.9b	€3.4b	€3.9b	€4.5b
Penetration	-	-	-	0.1%	0.5%	1%	3%	5%	10%
Products Sales forecast	-	-	-	€2.2m	€13m	€29m	€102m	€195m	€450m
Production capacity forecast (tons)				2,200	13,000	29,000	102,000	195,000	450,000
Goods in capacity forecast (tons)				11,000	65,000	145,000	510,000	975,000	2,250,000
Waste production forecast (tons)				8,800	52,000	116,000	408,000	780,000	1,800,000
Goods in Sales forecast				€1.1m	€6.5m	€14.5m	€51m	€97.5m	€225m
<b>Total Sales forecast</b>	-	-	-	<b>€3.3m</b>	<b>€19.5m</b>	<b>€43.5m</b>	<b>€153m</b>	<b>€292.5m</b>	<b>€675m</b>

Table 29 - Sale forecast for the BioICEP business operation

#### Financial Projections:

Year	Y-4	Y-3	Y-2	Y-1	Y1	Y2	Y3	Y4	Y5
	2021	2023	2024	2025	2026	2027	2028	2029	2030
Sales forecast	-	-	-	€3.3m	€19.5m	€43.5m	€153m	€292.5m	€675m
Costs									
Cost of production				0.55m	3.25m	7.25m	25.5m	48.75m	112.5m
Cost of waste				0.88m	5.2m	11.6m	40.8m	78m	180m
Cost of overheads	0.1m	0.2m	0.3m	0.5m	2.9m	6.5m	23m	44m	101m
Costs forecast				1.93m	11.35m	25.35m	89.3m	170.75m	393.5
Profit forecast				1.37m	8.15m	18.15m	63.7m	121.75m	281.5m
Investment	0.5m	1m	2m					10m	
<b>Balance</b>	<b>-0.6m</b>	<b>-1.8m</b>	<b>-4.1m</b>	<b>-2.73m</b>	<b>5.42m</b>	<b>23.57m</b>	<b>87.27m</b>	<b>199.02m</b>	<b>480.52m</b>

Table 30 - Financial projections for the BioICEP business operation

These financial projections highlight the potential for a highly lucrative commercial operation based on the outputs of the planned BioICEP developments. Further business models will be investigated as part of WP7, including options to licence developed enzyme cocktails and microbial consortia to different global customers.

The BioICEP consortium will aim at creating a spin-out company, which, from 2021 onwards, will develop the above business model further, will operate BioICEP's innovative processes and will commercialise the project's expected products and services. It is envisaged that a **Phase 1 feasibility assessment under the SME Instrument** will, during BioICEP's lifetime, be sought and performed. Following the confirmation of both the technical and economic viability in Phase 1, **Phase 2 of the SME Instrument** will be sought and performed having considered the feasibility assessment findings.

As seen in Table 31 above, the BioICEP business model's financial projections demonstrate the significant potential of the BioICEP innovative technology. While initial considerations for pre-commercialisation and operations ramp-up are taken (overheads and investments from Year -4 to Year -2 totalling €4m), a profit of near €300m by Year 5 of operations is expected, which would give an estimated balance of near €0.5bn by Year 5 and would allow for significant re-investment into technology improvement and growth into other markets, such as high-value chemicals. There are opportunities to set up multiple BioICEP plants localised at EU, China, and global plastic waste stockpiles.

Changing consumer behaviour and need for sustainability are seen as very attractive trends for investors. **BioICEP** will closely liaise with the cleantech investment community, as cleantech is currently one of the most important investment categories for private equity and venture capital investors.

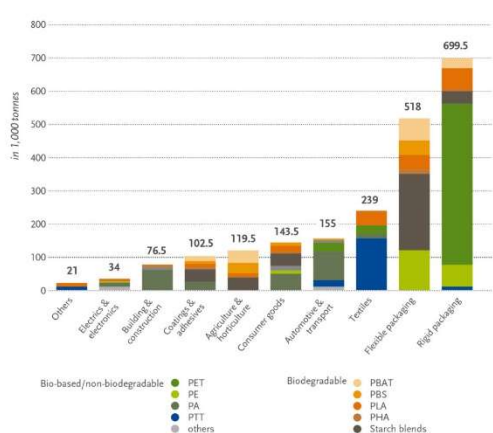
### Figures by market segment to assess the financial projections stated in the business model.

Here figures by market segments are provided for the primary sectors targeted by the project: 1) packaging (which is broken down into rigid and flexible packaging), 2) 3D printing filaments and 3) biosurfactants markets. Packaging remains the largest field of application for bioplastics with almost 65 percent (1.2 million tonnes) of the total bioplastics market in 2018. Based on market data from Nova Institute (2018) and an estimated bioplastics value of €1,000 per ton, the following are the packaging market figures:

**1A) Bioplastics rigid packaging:** growth from €699.5M in 2018 to €822M in 2023 (see Figure A);

**1B) Bioplastics flexible packaging:** growth from €518M in 2018 to €680.5M in 2023 (see Figure B); giving a total *bioplastics packaging* estimated market value of €1.5 billion in 2023.

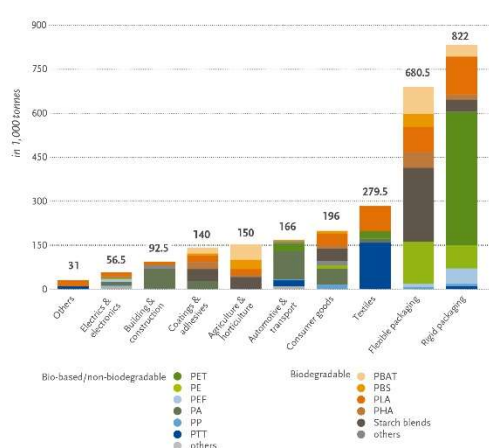
Global production capacities of bioplastics 2018 (by market segment)



Source: European Bioplastics, nova-institute (2018). More information: [www.european-bioplastics.org/market](http://www.european-bioplastics.org/market) and [www.bio-based.eu/markets](http://www.bio-based.eu/markets)

Figure A (Nova Institute figures for Global production capacities of bioplastics 2018)

Global production capacities of bioplastics 2023 (by market segment)



Source: European Bioplastics, nova-institute (2018). More information: [www.european-bioplastics.org/markets](http://www.european-bioplastics.org/markets) and [www.bio-based.eu/markets](http://www.bio-based.eu/markets)

Figure B (Nova Institute figures for Global production capacities of bioplastics 2023)

2) The third BioICEP high value added product is targeted at the 3D printing filament market segment, which in 2018 accounted for the largest share of the 3D printing plastics market and is projected to dominate

the market by 2023. The overall *3D printing market* is expected to grow from USD 9.9 billion in 2018 to USD 34.8 billion by 2024, at a CAGR of 23.25% (Markets and Markets, 2019).

- 3) Finally, *Rhamnolipids* are the fourth target bioproduct of the BioICEP project and are the growth-leading product within the global biosurfactants market, estimated at USD 4.20 Billion in 2017 and projected to reach USD 5.52 billion by 2022, at a CAGR of 5.6 % from 2017 to 2022 (MarketWatch, 2019).

Based on targeting 99.8% of the BioICEP sales to the bioplastics packaging market, 0.1% of the BioICEP sales to the 3D printing market and 0.1% of the BioICEP sales to the biosurfactants market, the figures presented in the following table are the projected total market values (incorporating the three target markets) and the forecast overall penetration rate, giving the total projected sale volumes for BioICEP:

Year	Y-4	Y-3	Y-2	Y-1	Y1	Y2	Y3	Y4	Y5
	2022	2023	2024	2025	2026	2027	2028	2029	2030
Bioplastics packaging market size	€1.3b	€1.5b	€1.8b	€2.1b	€2.4b	€2.8b	€3.3b	€3.8b	€4.5b
BioICEP productivity	-	-	-	99.8%	99.8%	99.8%	99.8%	99.8%	99.8%
3D printing market size	€23.0b	€28.2b	€34.8b	€42.8b	€52.6b	€64.7b	€79.6b	€97.9b	€120.5b
BioICEP productivity	-	-	-	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Biosurfactants market size	€5.5b	€5.8b	€6.1b	€6.4b	€6.7b	€7.1b	€7.4b	€7.7b	€8.1b
BioICEP productivity	-	-	-	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Total market size	€1.5b	€1.7b	€1.9b	€2.2b	€2.6b	€2.9b	€3.4b	€3.9b	€4.5b
Penetration rate	-	-	-	0.10%	0.50%	1%	3%	5%	10%
Products Sales forecast	-	-	-	€2.2m	€13m	€29m	€102m	€195m	€450m

Table 31 Projected total market values (incorporating the three target markets) and overall penetration rate forecast, giving BioICEP total projected sale volumes

These figures by market segment allow the feasibility of the financial projections stated in the business model to be assessed.

### 2.2.A.3. Strategy for generated research data management (Dissemination/Exploitation)

BioICEP project is fully aware of the open access to scientific publications as stated in art. 29.3. of the H2020 Grant Agreement. Open Access will be provided to peer-reviewed papers published by BioICEP's Partners as table 2.2.c indicates, either in an open access journal repository ("green" model) or for instance using the OpenAire repository. Depending on the results published, the selection of the best suited and higher impact repositories will be made on a case by case basis provided that the Intellectual Property Rights and Exploitation Board (IPREB) does not identify issues conflicting with the industrial exploitation of project developments. Technical press platforms, generic or specific, will be continuously used, as well as the BioICEP partners communication channels. According to the H2020 guidelines, a Data Management Plan (DMP) will be created by using the FAIR EC system. A deliverable (first version) will be provided in month 6 (Deliverable D8.2), and updated subsequently. Following the call recommendations, part of the modelling data (software data) will be shared in this DMP, based on the criteria of providing access only to those developed models developed at lab level, in intermediate packaging versions not 100% completed.



## 2.2.A.4. Strategy for knowledge management and protection (Exploitation)

A brief description of the background and foreground / exploitable knowledge, products or technologies identified by the consortium from BioICEP project during the preparation of this proposal for each partner is described in Table 32 below.

Participant	Background	Foreground/ Exploitation interests	Users/beneficiaries of the results	Means of Exploitation
1. AIT	Knowledge in plastic and bioplastic materials, processing, testing, functionalization, 3D printing, packaging applications	Pre-treatment processes to improve the accessibility of plastic waste for microbial degradation. New and Improved high sustainable bioplastics and bioproducts. Valorisation of waste plastic	Acquired knowhow. Waste treatment industries and industries using plastic as raw material, including packaging and additive manufacturing	Use of the knowledge generated in further projects. Improved industrial technical assistance services Technology transfer. Licensing to companies
2. ACT	Knowledge in plastic and bioplastic recycling and harvesting of degraded resources	Pre-treatment processes to improve the accessibility of plastic waste for microbial degradation	Plastic waste treatment and transition to bioplastics	Use of the knowledge i product and service development generating direct sales to end-users
3. AIM	Knowledge in plastic and bioplastic materials, processing, testing, functionalization, polymerization	Pre-treatment processes to improve the accessibility of plastic waste for microbial degradation.	Acquired knowhow Waste treatment industries and industries using plastic as raw material (eg plastic converters)	Use of the knowledge generated in further collaborative research in research and industrial projects. Technical assistance services. Licensing to companies
4. AVE	knowledge in optimizing and steering microbial processes, microbial molecular analysis and extensive experience with designing and constructing reactor setups at lab, demo or pilot scale.	Reactor design for PHA production.	Acquired knowhow. Waste treatment industries and industries using plastic as raw material (e.g. plastic converters)	Use of knowledge for product improvement and development. Direct sales generation
5. CUT	knowledge in plastic and bioplastic materials, characterisation and processing	Pre-treatment processes to improve the accessibility of plastic waste for microbial degradation.	Acquired knowhow	Use of the knowledge generated in further collaborative research in research and industrial projects. Technology transfer
6. iBET	bioengineering, wastewater treatment, nutrient removal, microbiology and modelling, including the use of microbial based processes for biopolymer production	New and Improved high sustainable bioplastics and bioproducts. Valorisation of waste plastic	Acquired knowhow Manufacturers using bioprocessing. Bioplastic and the bioproduct industrial chain	Further collaborative research with industrial partners. Improved technical assistance services. Licensing to companie.
7. IMGGE	Knowledge in microbial strain isolation and functional screening, recombinant protein expression, directed evolution of the proteins, strain improvements, bacterial fermentations for biopolymer production	Enzymatic digestion of pretreated plastic waste. Acceleration of target microbe and enzyme detection and performance enhancement	Acquired knowhow Waste treatment industries and industries using plastic as raw material	New and Improved high performance biocatalysts and bioprocessing microbes for further project applications. Technology transfer. Licensing to companies
8. LIT	Knowledge in microbial strain isolation and functional screening. Microbial	Microbial degradation and synthesis	Acquired knowhow. Waste treatment industries and industries using plastic as raw material	New high performance biocatalysts microbes for further project applications. Technology transfer. Licensing to companies
9. LOG	Polymer and biopolymer packaging	Integration of new bioplastics within rigid food packaging	Acquired knowhow. Sustainable packaging industries	Use of knowledge for product improvement and development
10. MLS	Knowledge in microbial strain isolation and functional screening and microbial consortia development	Microbial degradation and waste water treatment	Acquired knowhow Waste treatment industries and industries using plastic as raw material	Use of knowledge for product and service improvement and development. Direct sales generation
11. NTUA	Knowledge in microbial strains isolation and screening, fungal esterases and other hydrolytic enzymes, recombinant protein expression, polymer surface modifications	Enzymatic digestion of pretreated plastic waste. Biocatalysis performance enhancement	Waste treatment industries, Biorefineries Waste treatment industries	Use of the knowledge generated in further projects. Technology transfer. Licensing to companies

Participant	Background	Foreground/ Exploitation interests	Users/beneficiaries of Results	Means of Exploitation
12. TCD	Knowledge in plastic and bioplastic materials, processing, testing, functionalization,	Pre-treatment processes to improve the accessibility of plastic waste for microbial degradation. Compostable plastics. Valorisation of waste plastic	Waste treatment industries and industries using plastic as raw material, including packaging and additive manufacturing	Use of the knowledge generated in further projects. Technology transfer. Licensing to companies
13. BIT	Knowledge in microbial strain isolation and functional screening, recombinant	Screen microorganisms to obtain highly efficient plastic degradation strains	Acquired knowhow Waste treatment industries and industries using plastic as raw material	Use of the knowledge generated in further projects and service development
14. CAS	Protein engineering techniques. Recombinant microorganisms High throughput screening tools	Acceleration of target microbe and enzyme detection	Acquired knowhow Waste treatment industries and industries using plastic as raw material	Use of the knowledge generated in further projects and service development
15. SDU	Microbial based processes for biopolymer production. Knowledge in microbial strain isolation and functional screening	Bioprocess development for bioplastics production	Acquired knowhow Waste treatment industries and industries using plastic as raw material	Use of the knowledge generated in further projects and service development

Table 32 - BioICEP partners background, foreground intellectual property and exploitation interests

The key exploitable results will be presented in a roadmap for the commercialisation of the technologies and process developed. The business plan, to be prepared in WP8, will build on the information in the outline business plan presented above and identify the optimal route to market for the BioICEP processes and products.

Each partner has communicated clear exploitation expectations which will be planned in greater detail during Task 8.3. A detailed business plan will be developed in order to provide the details on how the project's results will be used in commercial exploitation activities. The business plan will detail how each of the BioICEP project outputs will be used in commercial exploitation activities for business growth and competitiveness, including elements such as, but not limited to:

- ▣ Purpose, main features and benefits of each technology or product;
- ▣ Innovative aspects in comparison with existing and competing technologies and products;
- ▣ Summary of need for further R&D activity (and implied risks);
- ▣ Standardisation, approval and policy implications involved;
- ▣ Collaboration required for exploitation (technology transfer activities);
- ▣ Identification of the potential customers and the factors that affect their purchasing decisions;
- ▣ Features of the target market (size, growth rate, share that the technology/product could reach);
- ▣ Any factors likely to drive a change in the market such as legal, technical, and commercial barriers;
- ▣ Other technologies likely to emerge in the near future;
- ▣ How each project partner/beneficiary that is entitled to the technology exploitation is positioned (or should be positioned) in the market;
- ▣ Competing businesses/applications/technologies; and
- ▣ Further research opportunities for building on the project results and for realising transfer of the technology to other applications (which will be picked up in the research roadmap).

## 2.2.B. Communication activities

BioICEP consortium members will facilitate dissemination of outreach material and results through their established institutional websites, contacts, newsletters, and strategic boards throughout Europe (such as the European Technology Platform on Bio-based Products) and worldwide.

The dissemination and exploitation (DE) manager and project manager (PM) will publicise BioICEP through a dedicated BioICEP website <https://www.BioICEP.eu> which will serve as an open access portal to disseminate and transfer knowledge, data and research activities and expertise. The BioICEP website will host both internal (restricted) and public information and will facilitate communication, report and data sharing among consortium members. The public portion of the BioICEP website will publicise less sensitive, non-confidential material to the greater research and industry community (public). Liaison with the general public as part of



BioICEP will serve to inform the public of the importance of project objectives at a societal level. BioICEP consortium members will update the DE manager and PC with non-sensitive information which will be collated and reviewed prior to posting. The DE manager and PM will then release non-sensitive information to the general public via the project website, local and international newspapers radio and television communications. Social media updates will coincide with conferences, policy reviews, trade shows, and key BioICEP dissemination events.

Reviews and publications arising from the BioICEP projects will be submitted peer reviewed journals such as: *Polymer Reviews, Journal of Molecular Pharmaceutics & Organic Process Research, Natural Products Chemistry & Research, Journal of Molecular Pharmaceutics & Organic Process Research, Journal of Reinforced Plastics and Composites, Polymer - Plastics Technology and Engineering, Plastics, Rubber and Composites, Journal of Elastomers and Plastics, Plastics, Additives and Compounding, and Current Opinion in Microbiology.*

The main communication strategies have been designed to ensure that the commercial impact of the project is not compromised. Therefore, the project results to be communicated will be split into:

- Publicly available information that will be widely communicated; and
- Confidential information which will not be communicated outside of the consortium.

The communication measures will be outlined in the Consortium Agreement before the project start. An internal consortium review protocol will be agreed for dissemination activities, to allow consortium review prior to the disclosure of any dissemination contents, to safeguard IP rights, confidentiality and commercial interests of the project partners. Plans will be discussed at steering committee meetings to ensure that the dissemination is in the interest of all the partners.

In addition to communicating about the project and results, BioICEP also intends to share all the publicly communicable deliverables within the scientific and industry communities related to our end-users. Increasing the awareness of the innovation results of the project is a crucial task. Therefore BioICEP's communication strategy is designed very carefully. It will provide a regular flow of information rather than occasional ad-hoc announcements since this will contribute to the establishment of recognition and increase the opportunities for publicity.

BioICEP's strategic dissemination plan answers: *WHO* (target audiences), will receive *WHAT* (key messages), *HOW* (communication channels), and *WHEN* (implementation and time planner).

One of the main aims of this plan is to provide a guide to manage the dissemination activities in order to gain international visibility and repercussion. All dissemination activities must be approved by the consortium according to the provisions set in the CA and the GA. While a draft communication plan is given below, a more detailed plan will be elaborated at the beginning of the project (D8.2) and updated through the project lifecycle.

#### WHO AND WHAT: Target groups and key messages

**WHO:** Plastic producer industries, microorganisms' industries, bioplastic manufacturer Industry in Europe and China and technology providers

**WHAT:** Plastic suppliers and producers are one important target group, since this project's results expect to alter completely the market of plastic and the industries which relate with this material. Potential end users of the project's results are also related to biotechnology industry. The dissemination strategy will include activities undertaken during the project's duration (such as organizing workshops or attending fairs and events) aiming at the increase of the project's awareness, its objectives and its foreseen results during the project's lifetime and after its ending aiming at fostering exploitation of the project's results. Special focus will be set on the dissemination of technical and economic results arisen from the validation phases, strengthening the confidence of the aforementioned target groups in the pre-treatment technologies.

**WHO:** Technical experts, researchers and scientific community in Europe and China

## WHO AND WHAT: Target groups and key messages

**WHAT:** The scientific community and technical experts are also key audience to enlarge a further replication and dissemination of this project results. Experts will be given open access to the technical publishable results of the project in relevant journals of the biotechnological sector. Presence at conferences through dedicated keynote speeches, conference proceedings, publications in scientific and technological specialized magazines, and peer-to-peer communication will further support dissemination towards this target group. The main messages to deliver are the technical results, innovation and progress beyond the state of the art on the R&D lines proposed, and future challenges that emerge during the project execution.

**WHO:** Policy makers, authorities and public bodies in Europe and China

**WHAT:** the European Commission, regional local authorities, permitting bodies, and municipalities with competences in the field of this project are essential for the establishment of a new policy. The messages to send them are the market potential evaluation, lessons learned, and a socioeconomic analysis, generating in this way a contribution to EU and Chinese policies and directives and to the EU and China goals achievement. Local economy fostering as well as waste streams reduction and improvements in circular economy are key. In this way, the raising of awareness and the acceleration of regulatory-related processes is expected. It is also important to identify regulatory aspects at national or regional levels which could reduce the project dissemination potential.

**WHO:** General public. In particular young audience will be targeted as the future global citizens

**WHAT:** Citizen organizations and individual citizens (especially young audiences) are also a potential audience for dissemination. Entertaining short stories and documentaries, facts about environmental footprint reduction, employment generation, increasing European competitiveness and reducing external dependency will be the key messages to be sent to the general public, aiming to reduce the existing resistance and motivating early adopters. Four online training webinars will be launched and promoted in schools and high schools to disseminate the project outcomes among young citizens. The results of the project will also be disseminated to the wider general public, enabling European citizens to understand the project innovation, its achievements and the lessons learnt during it, trying to raise the general public's awareness in plastic waste management issues.

Table 33 WHO AND WHAT: Target groups and key messages

While the who and what is given above, the development of the communication plan will be done in parallel with the creation of a promotion and marketing strategy to engage a relevant group of stakeholders aware and interested in this project. This action aimed at the highest impact possible (see "excellent impact" below) will be sustained during the whole project execution, broadening the permanent audience this project will have. This will be crucial to achieve dissemination and impact goals.

## HOW AND WHEN: Most relevant dissemination channels and methods used in the project

There are primary and secondary communication channels in use to spread developments and project results. The primary ones will be formal communication channels, such as a project web site, dedicated communication material, local stakeholder meetings, podcasts, and similar. The secondary communication channels are related to less measurable things as networking and broader public relations activities of this project partners.

### HOW AND WHEN: Most relevant dissemination channels and methods used in the project

#### LOGO (M1)

The logo of this project will be selected by the consortium from a number of designs proposed, considering that it should be easily used in printouts, projected slides and on the web. Before the selection of the logo, a branding analysis will be done in WP8/D8.1, in order to ensure that no copyright is affected and higher visibility is achieved by means of effective marketing measures.

#### WEBSITE CREATING AND UPDATING - WP8 / D8.1 (M3)

The website will be the main communication tool for the project, where all the dissemination materials will be published in a timely manner. The website will be an interactive environment that will give access to all the publishable developments of the project, including a link for its downloading, the status of the project and the final results. The website will be published in English and Chinese and its structure will differ from traditional project web pages, which usually users find difficult to access relevant content. It will give a very direct link to the main results and to the hottest project news. Besides, this website will be a link to the objectives, partnership, activities and events related to the project, and it is planned to give access to all the aspects regarding the new technologies, best practices and recommendations for plastic pre-treatments gathered from the project development. Moreover, a private part (Intranet) will provide a collaborative working space for developing the project and schedule the work in an efficient manner. Contributions from the partners will be highly important to maintain the project website's relevance, in order to improve the website positioning in search engines and to reflect an active attitude to Internet users. In addition, partners are asked to link their website and platforms to the website of this project.

## HOW AND WHEN: Most relevant dissemination channels and methods used in the project

Key Indicators	Poor Impact	Good Impact	Excellent Impact
Web page visits per year	< 2,000	4,000 - 8,000	> 8,000
Material Downloads	< 500	500 - 1,000	> 1,000

### PUBLICATIONS (throughout the project)

The consortium partners will publish the results they consider relevant, mainly related to biological, material and engineering sciences (according to the IPR protection strategy and to the GA and the CA) in the scientific literature, dedicated journals and magazines. Results will be also published in partners' websites and the project's newsletters. Additionally, sectoral platforms and associations will also receive information from the project. Moreover, the new concept and developments expected in this project can derive in the consecution of new patents. The patents are also public knowledge published in the international databases. The task leaders and deliverable responsible are asked to take into account the date of publication of the deliverables in order to generate a publishable version of the corresponding deliverable (WP8/D8.4).

**Pre-selected scientific Journals:** *Polymer Reviews, Journal of Molecular Pharmaceutics & Organic Process Research, Natural Products Chemistry & Research, Journal of Molecular Pharmaceutics & Organic Process Research, Journal of Reinforced Plastics and Composites, Polymer - Plastics Technology and Engineering, Plastics, Rubber and Composites, Journal of Elastomers and Plastics, Plastics, Additives and Compounding, and Current Opinion in Microbiology*

Key Indicators	Poor Impact	Good Impact	Excellent Impact
Number of papers submitted	< 20	20 - 60	> 60
Technical project publications downloads	< 15	15 - 50	> 50

### FINAL CONFERENCE, WORKSHOPS AND EVENTS (at least one every year)

This project will be presented in a number of relevant international forums and events related with its scope, such as conferences, exhibition fairs, etc. Regarding the European forums, this project will take advantage of the existing relation of its partners, tackling those forums, associations, and platforms in which the consortium has an active role. Contacts with target groups will also take the form of workshops, set up by the project on different locations across EU (WP8/D8.4). The objective is to discuss project results and receive inputs from outside. BioCEP project will organize or participate in 2 open workshops / 1 conference (dedicated on or in collaboration with larger initiatives) in which the results will be delivered. At the conclusion of the project, the consortium will organize one international conference where results will be explained (WP8/D8.6). Moreover, at this final conference the replication and exploitation strategy beyond this project and the real expectations that has the consortium concerning the new solutions developed will be presented. The Chinese partners will also host a workshop to disseminate the project results and strengthen scientific cooperation between Europe and China (WP8/D8.5). The following audiences will be targeted: industries, universities and scientific partners, local authorities, and policy makers. During the event a matchmaking event to maximize the cooperation in future project and direct business will be organised.

**Pre-selected Conferences and Events:** *International Conference on Bio-based Materials, European Bioplastics Conference, Annual Circular Economy Stakeholder Conference, International Conference on Waste Management and Technology, World Conference on Waste Management, International Conference on Technologies & Business Models for Circular Economy, International Symposium on Biopolymers*

Key Indicators	Poor Impact	Good Impact	Excellent Impact
Number of conference presentations	< 6	6 - 8	> 8
Number of workshops for policy makers	< 3	3 - 6	> 6

### PRESS MEDIA (throughout the project)

One of the targets addressed by the project dissemination is general public, and the corresponding main channel is media. Partners are encouraged to contact media (either general or specialized) in order to increase the project's visibility and to spread the activities and results. This will be achieved by: 1) The dissemination of a press release and 2) inviting media to the main events celebrated during the project. A press kit will be developed to help partners as part of the communication plan (WP8/D8.2) in the elaboration of their press releases, or to help journalists on the elaboration of articles about the project. Containing: 1) **Writing identity of the project:** Descriptions of the project to be used for different requirements; 2) **Press release:** Information of the project on press format with more detailed information than the written identity; 3) **General presentation:** Description of the project on PPT format; 4) **Tweetable facts** concerning the project; 5) A list of **frequently asked questions:** Several questions and answers for general public; 6) **Previous press releases & media impacts:** Examples of previous press releases and their respective impact on the media; and 7) **Copyright free Photographs:** Images sent by partners to be used by any person. Partners are asked to send all the appearances of the project in the press (TV, newspapers, radio, webs. etc.) to the dissemination manager, who will gather all the contributions for the elaboration of a report gathering the results of the dissemination task and will form part of the final report (WP1/M1.1).

Key Indicators	Poor Impact	Good Impact	Excellent Impact
Number of press releases	< 10	10 - 15	> 15
Mails out and newsletters	< 200	200 - 350	> 350

### LEAFLETS & POSTERS (throughout the project)

Graphic materials will be developed to promote the project at selected events providing general information and preliminary results, addressing both technical and non-technical audiences. During the project execution, two versions of this material

## HOW AND WHEN: Most relevant dissemination channels and methods used in the project

will be released, firstly with a general presentation of the project and at the end of the project gathering the results in a leaflet and dissemination poster. Both leaflet and poster will be uploaded to the website and will be available for download to any visitor to the project website. The printable versions will be uploaded in the intranet of the project, as it will serve also as support document for fairs, congress, forums, and workshops.

### COOPERATION WITH OTHER PROJECTS (throughout the project)

This project will outline and enlarge the previous list of European projects related to plastic waste management and bioplastic production and previous BIOTEC and BBI calls in order to find synergies and collaboration opportunities. Additionally, any partner participating or cooperating in national or international projects related to the lines and impacts of this project will notify the Project Coordinator and the Dissemination manager. This will enable the discovery of synergies with other projects to establish cluster participation in events and publications, as well as to multiply the dissemination potential of the public website by sharing news and links.

Key Indicators	Poor Impact	Good Impact	Excellent Impact
Number of projects	< 5	5 - 10	> 10

### SOCIAL & PROFESSIONAL NETWORKING DEVELOPED (throughout the project)

Networking opportunities allow project partners to learn from each other, discuss common issues and get feedback on their work. These kinds of meetings also provide a great chance to carry out an effective dissemination of the project inside and outside the consortium. Instead of using an own account for the project, it has been outlined that it is better to disseminate the project's features from the project members accounts, benefiting from the popularity of these entities and then, enhancing the project's image.

In this sense, the partners will evaluate new routes for dissemination using social and professional networks, such as LinkedIn, Twitter, Facebook, or YouTube, to create discussion, solve doubts and detect future industrial investors from other cities in Europe.

**Pre-selected Associations / Platforms / Networking:** European Bioplastics e.V., European Composites, Plastics and Polymer Processing Platform, CHINA BIODEGRADABLE AND BIOBASED GROUP (BMG), CHINA DEGRADABLE PLASTIC COMMITTEE (DPC), DECHEMA

Key Indicators	Poor Impact	Good Impact	Excellent Impact
Collaboration agreement with relevant networks	< 2	2 - 4	> 4

Table 34 - HOW AND WHEN: Most relevant dissemination channels and methods used in the project

### Communication activities beyond the project's lifetime:

The communication activities will not end when the project's final deliverables are submitted. Only communications activities within the project's lifetime will be charged to the project. The main tools and actions, which will remain beyond the project to enhance dissemination impacts, are the following:

- **Website maintenance:** The project's website and its deliverables are envisaged to be maintained for 5 years after the end of the project by continued maintenance from AIM. This will be the main repository of information for the consortium and its maintenance will be the responsibility of the dissemination WP leader;
- **Participation in forums:** BioICEP consortium partners are committed to show results at conferences and trade fairs related to the project targets during and after the end of the project.

END OF SECTION 2

PART B

SECTION 3

IMPLEMENTATION

# SECTION 3 - IMPLEMENTATION

## FRONT PAGE PLACEHOLDER



**Work Plan Overview**

BioICEP is an innovative merger of cutting-edge technologies designed to surpass current state of the art and provide a route to a sustainable circular economy for plastics, where current approaches fail.

The work plan is constructed to foster strong collaboration between the consortium partners across the content of Europe and China and ensure the project objectives and deliverables are met. WP 1 is dedicated management and coordination of the project, while the final WP focuses on the dissemination and communication of the project results and outputs. There are six technical WP's tasked to complete the ambitious work proposed with strong feedback loops and inter-partner participation planned in order to optimise the results and performance of the target technologies. This close technical interoperation will promote effective inter-disciplinary knowledge and propel innovative solutions to overcome obstacles and boost the project technical outputs.

The first four technical work packages (WP2, WP3, WP4, and WP5) are assigned to developing BioICEP's triple action depolymerisation technology to degrade more than 20 % of mixed waste recalcitrant plastics. WP 6 will valorise the outputs of WPs 2-5 producing high demand bioproducts.

The objective of WP 2, led by TCD, will perform stage one of the depolymerisation using proprietary and novel mechano-biochemical treatments of mixed plastics waste. WP 2 will provide samples of pretreated plastic substrates to the biocatalytic and microbial strain degradation WPs and carry out further process refinements based on feedback received. WP2 will also blend new compatibilised bioplastics from the WP 6 output bioproducts for the preparation of filaments for 3D printing.

Augmented biocatalysis, the second stage of the depolymerisation process is developed in WP3, led by IMGGE. Here a number of novel approaches and new booster technologies are deployed to develop enzymatic cocktails for high mixed plastics degradation. WP4, led by LIT, is dedicated to the discovery and generation of novel strains with boosted plastic degradation capacities while WP5, led by MLS, optimises synthetic and enriched natural plastic degradation microbial communities. In WP6, led by iBET, the fermentable carbon outputs of the triple depolymerisation process are bioprocessed to form in-demand bioplastics and bioproducts.

**Elaboration with added details, on the objective of developing microorganism communities**

Building the stable and cooperative microbial communities is relying on the fact that the vast diversity of microbial metabolic capabilities offers opportunities for the production and the exchange of specific metabolites between two or more microbes that can be mutually beneficial. Such microbial communities consist of member organisms that, together, are more robust to environmental challenges, exhibit reduced metabolic burden due to a division of labor and exchange of resources, possess expanded metabolic capabilities relative to monocultures, and can communicate (chemically or physically) between species.

Development of the robust microorganism communities will allow diversification of biochemical roles in breaking down complex substrates, such as mixed plastic waste. Furthermore, synthetic communities will be further engineered with increased robustness through interdependencies and spatiotemporal control.

For the initial design of the microbial communities for mixed plastic waste degradation the Metabolic Tinker (<https://omictools.com/metabolic-tinker-tool>), will be used to predict and design new metabolic interactions between synthetically engineered microbial consortia. In addition, number of biodegradative databases (information related to biodegradation of chemicals including xenobiotics-degrading bacteria, metabolic degradation pathways of toxic chemicals, enzymes and genes involved in the biodegradation) will be mined for the information including the University of Minnesota Biocatalysis/Biodegradation database (UM-BBD), a database of biodegradative oxygenases (OxDBase), Biodegradation Network-Molecular Biology database (Bionemo), MetaCyc, and BioCyc. Based on in-silico information and phenotypic screens, microbial strains will be selected to build communities with specific functions (surface modification or breakdown of single or multiple plastic substrates). Created communities will be from relatively simple (2-3 strains for enhanced breakdown of single substrate or enhanced production of target products) to quite complex (10-15 strains to successfully deal with mixed substrate) and some would be naturally occurring communities (number of essential constituents strains will be determined during the study).

Therefore, this bottom-up study of synthetic communities will yield a better understanding for natural microbial ecology by systematically evaluating individual parameters in a controlled environment in an iterative design-test-learn cycle. Key principles that are already been well described in the literature for establishing the microbial communities will be followed to enhance cooperation, avoid 'cheating', and promote non-metabolic stabilizing interactions.

The strains selected to form the communities will express enzymes such as laccases, manganese peroxidases, lignin peroxidases as well as hydroquinone peroxidases. Synthetic, plastic degrading communities will be established after determining optimal plastic breakdown potential of existing strains and communities and of newly discovered communities from **WP4** using the principles described by (Johns et al., 2016). Synergistic effect of microbial communities on various single plastic materials has been reported in the literature (Park and Kim, 2019, Roy et al., 2008, Satlewal et al., 2008, Yang et al., 2015). Novel microbial communities from petroleum contaminated sites will be isolated and functionally screened (Frioux et al., 2018) against single and mixed plastic materials using standard methodologies established in **WP3** and **WP4**. Synthetic communities will be analyzed for community stability using taxonomic sequencing as well as its potential to breakdown mixed plastic waste streams into plastic monomer constituents.

The special attention would be placed into optimization of spatiotemporal organization of the microbial communities designed for the degradation of mixed plastic waste. Spatial assortment of cells creates locally heterogeneous subpopulations with varying resource availabilities that strengthens local interactions, avoids global resource competitions between species, and improves resilience to environmental stresses such as accumulation of toxic byproducts. Several general approaches will be explored to build spatially defined microbial communities by organizing the physical environment and/or patterning specific community structures. For example, microfluidic and microwell devices will be used to build microbial communities where individual species are grown in separated chambers that allow metabolites to exchange freely, but restrict physical contact between cells or they will be arranged in defined patterns on two-dimensional surfaces as resilient biofilms.

#### In-situ biosensors

With very large numbers of microbes to sort through, it is a big challenge to hunt for the most promising strains in the bacterial haystack, especially for the function of recalcitrant plastic breakdown. Therefore, the development of the highly sensitive biosensors to respond to low levels of recalcitrant plastic degradation would immensely alleviate the screening procedures and allow selection of good strains. These biosensors can be further used to monitor the performance of the developed consortia.

#### **Verification of main project results in a non GMO Pilot Plant.**

WP7, led by AVE, implements the integrated bio-depolymerisation and bio-synthesis processes in a pilot plant set up. The environmental impact and commercialisation roadmap for the BioICEP technology will also be developed in WP7. The pilot plant is restricted from using GMOs solely due to the higher costs that would be incurred, and which cannot be covered by the available budget within the project. With further research investment, a GMO compliant pilot plant, which incorporates GMOs developed within the project, can be readily established. As stated in Table 5. of part B Annex 1, the purpose of the modular pilot plant is as a "Demonstration of all in one mixed plastic waste depolymerisation and biosynthesis of high value bioproducts" Given the financial restraints, and using the merits of the modular nature of the pilot plant, the best non-GMO performing microbiomes will be operated to demonstrate the BioICEP technology at TRL5/6. The stated project objective is that "The pilot plant will be operated to degrade at least 20% of mechano-biochemical pretreated mixed recalcitrant plastics and subsequent bioprocessing into PHB/rhamnolipid/nanocellulose bioproducts". Within the project for the purposes of non-GMO pilot plant operation, scaled up wild-type strains and consortia composed of these will be used as non-GMO constructs. The consortium is confident that we are not fully dependent on GMO's to achieve and match the objectives of the proposal. A fully GMO operational system will be included within the project business model. The GMOs will be used at lab scale and based on the laboratory results and performance of the GMO's developed within the project and the significant improved performance that can be projected on their inclusion in the pilot plant operation will be analysed and documented. This will facilitate the full potential operational performance of the BioICEP technology for further development and commercialisation beyond the project.



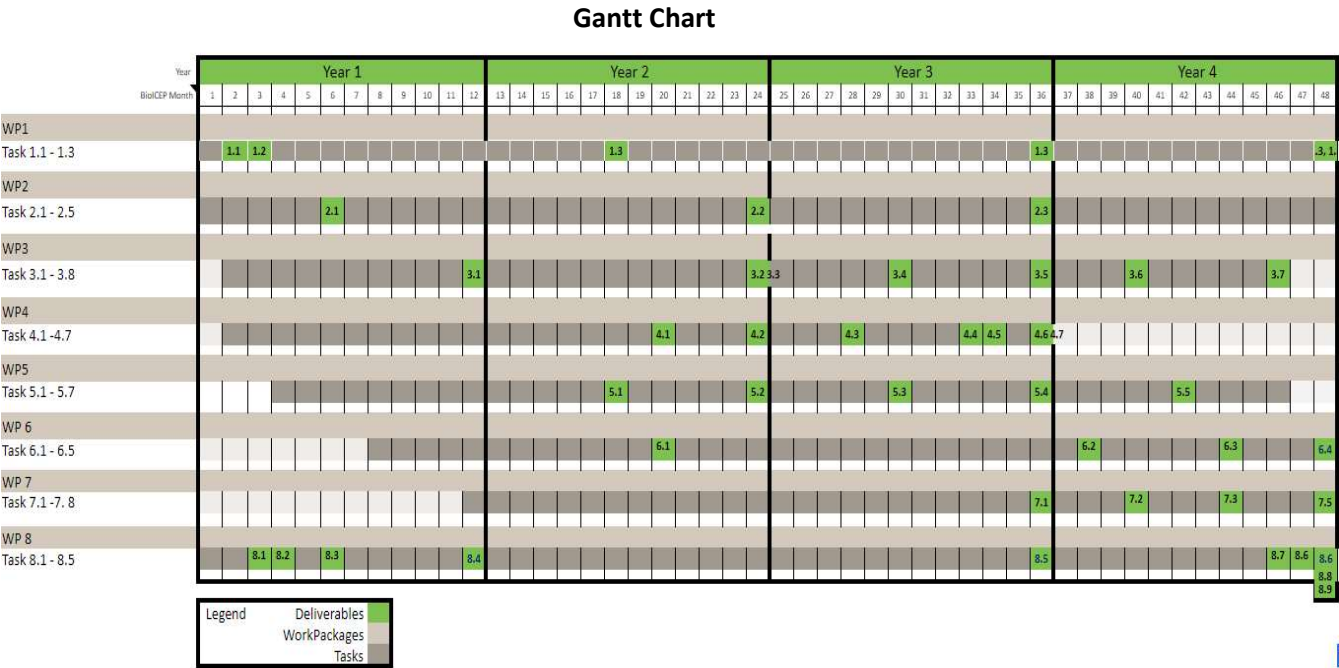


Figure 10 - Gantt Chart

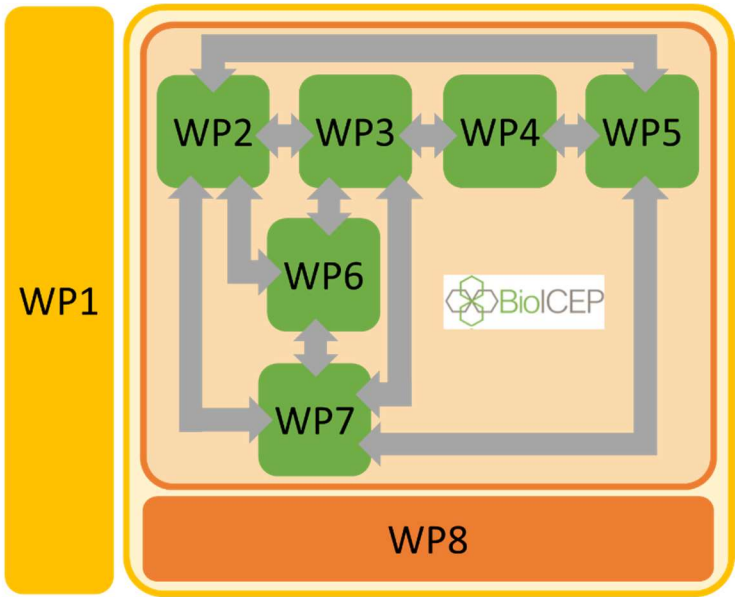


Figure 11 - PERT Chart

**3.2. Management Structure and Procedures**

One of the key elements of success for international projects is a professional project management, comprising a strong team for coordination, administrative, and financial management. In order to ensure close collaboration and timely preparation of the project results, the fifteen project partners of BioICEP apply a hierarchical and robust management structure. Figure 12 gives an overview of these bodies and their relationship. An EU-China engagement officer role is included to support the effective relationship between EU and Chinese partners. This role will facilitate good communication including accurate translation and navigation of cultural differences to ensure the best management, scientific and dissemination outcomes for the project.

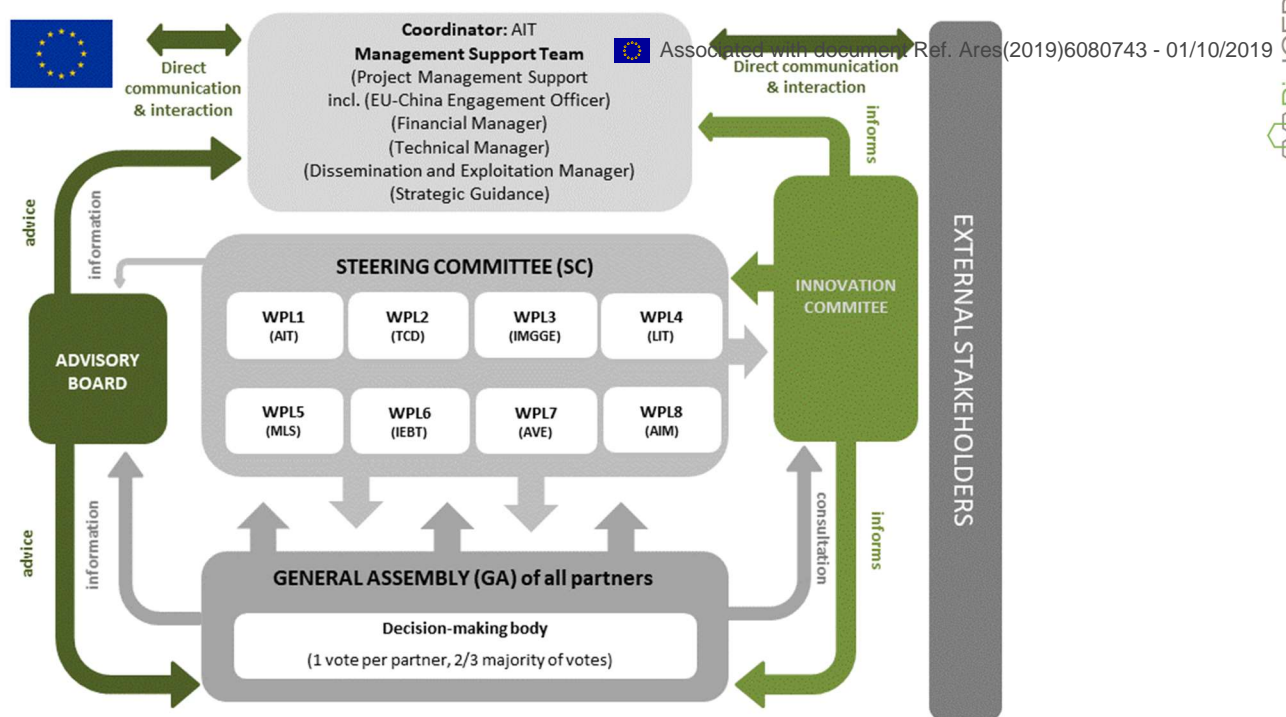


Figure 12: Organigram of BioICEP showing the hierarchical structure with the Coordinator on top. The arrows indicate responsibilities and flow of communication

The management structure consists of a Management Support Team, Steering Committee, General Assembly, the External Advisory Board and an Innovation Committee. Each of these bodies are explained in the sections below.

### 3.2.1. Project Bodies

#### Coordinator

AIT as the **Coordinator** is the legal entity acting as the intermediary between the Parties and the EC. The Coordinator, in addition to its responsibilities as a Party, will perform the tasks assigned to it as described in the Grant Agreement and the Consortium Agreement. Dr Margaret Brennan Fournet will act as the project coordinator. She has 15 years' experience in coordinating projects as demonstrated also from her CV in Section 4. Within BioICEP, the Coordinator ensures the proper implementation of all project tasks and management procedures and is responsible for

- Distributing EC funds to the beneficiaries according to the Grant Agreement;
- Monitoring compliance of treaties and partner's obligations, e.g. IPR, Consortium Agreement;
- Applying risk-management;
- Chairing the Steering Committee; and
- Leading the bi-annual meetings, proposing decisions, providing minutes.

There is a **Management Support Team** that will be supporting the Coordinator with the day-to-day running of the project. The management support team will consist of:

- A project manager, experienced in the operation of H2020 projects will be hired to assist the coordinator with various tasks.
- Sarah Keegan will be responsible for the financial management; she has over 15 years' experience at AIT in that role.
- AIT will nominate a technical manager. As a key person in the development of the BioICEP technical concept their role will be to support the coordinator with the technical management of the project.
- Dr. Ana Planca from AIM will be the dissemination and exploitation manager. She has more than 15 years' experience in similar roles.
- Dr. Patrick Murray, who has been working at LIT for 30 years in international projects and as an expert for the EC already from FP7, will be providing advice and support to the coordinator and the Management Support team as required.

The Partners will supported enabling them to understand and fulfil their roles including setting-up appropriate accounting systems – Financial and technical reporting and regular internal reporting with close monitoring in order to fulfill their contractual obligations

### Steering Committee

The Steering Committee consists the eight Work Package Leaders, which are all EU beneficiaries and it monitors the effectiveness and efficient implementation of the project. There will be monthly video-conferences of the steering committee where updates about the progress of each work package will be provided. In addition to scientific and technical issues, the committee will support the coordinator on issues such as finance, IPR including use of background, deviation of the work plan including budget, and other issues that may arise.

### General Assembly

The General Assembly will consist of one representative from each consortium member which will include the three Chinese international partners. It will be the ultimate decision-making body of the Consortium and each partner has one vote with cumulative vote of three for the international partners out of a total of 15. The Project Coordinator will chair the General Assembly. The General Assembly will be in charge of the overall direction and major decisions with regards to the project. All rules governing the General Assembly such as meetings, voting rules and quorum, veto rights etc. are clearly defined in the Consortium Agreement.

### Innovation Committee

The project will put in place an Innovation Committee (IC). The Innovation Committee will be chaired by AIT and will be composed by an IPR expert from each of the participant organisations including the three international partners. This committee will be consulted by the other project bodies on any IPR issues relevant to the project. The Innovation Committee is an advisory committee with no votes allocated to the members. Therefore, the final decisions will be made by the partners owning the Foreground. The IC role will consist of:

- ▣ Assisting partners in identifying foreground IP and innovative tools that could be subject matter of protection;
- ▣ Providing advice on the determination of foreground ownership, management of joint ownership, granting of access rights, freedom to operate and patentability, and choice between patent and other protections;
- ▣ Establishing and adapting the exploitation plan of the project taking into account the new industrial and market opportunities;
- ▣ Managing internal and external opportunities and facilitate commercial agreements between partners aimed at promoting the exploitation of the project results;
- ▣ Coordinating and supervising the preparation of exploitation reports. Lead the preparation of business plans; including product definition, target customers, exploitation strategies, product life cycle, etc. Revise and update the business plans by incorporating feedback from the partners, potential customers and/or technical developments outside of the project;
- ▣ Monitoring the project progress to guarantee consistency between technical and marketing choices;
- ▣ Identifying market risks and opportunities with respect to the evolution of the technology, potential customers and / or existing and emerging competitors; and
- ▣ Managing IPR protection by means of the IPR protection plan.

The Innovation Committee will hold meetings back to back with the Project Progress meetings and the recommendations coming out from each meeting will approved by the General Assembly.

**Advisory Board** The Advisory Board will be set-up and consulted as described in Task 8.1. It will include representatives of the main stakeholders, as identified in Section 2.2. Here are given some examples of the potential Advisory Board entity:

- ▣ Example 1. Ji-Dong Gu, *Ph.D. Editor-in-Chief* International Biodeterioration & Biodegradation
- ▣ Example 2. John Wallace, *Plastics Ireland* Chairman - Irish Medtech Association
- ▣ Prof. Angel T. Martinez Project coordinator from the Biological Research Centre

Table 35 – BioCEP Dissemination and Exploitation Board

### Dissemination and Exploitation Board

BioICEP will nominate a **Dissemination and Exploitation board** (DEB) chaired by the Project Coordinator (PC) to facilitate outreach and maximise the expected impact of the project. The DEB will appoint a dissemination and exploitation (DE) manager and will include all SME consortium members and the consortium IP specialist to ensure industry leadership and IP protection in all BioICEP dissemination activities. All BioICEP dissemination, exploitation, technology/knowledge transfer and IP activities to progress products to market will be coordinated by the DE manager and the PC, in consultation with the EU Project and Scientific Officer as outlined in the Horizon 2020 guidelines for Communicating EU research. DEB meeting outcomes will be relayed to the EU Project Officer, Scientific Officer, and members of the consortium. The international partners are not members of the DEB.

### 3.2.2. Decision making process

#### Consortium Agreement

All partners are obliged to sign the Consortium Agreement (*the DESCA3.0<sup>25</sup> template is the basis for the BioICEP Consortium Agreement*), which has to be in accordance with the objectives of the proposal and with the Grant Agreement. The Consortium Agreement regulates the interaction between the beneficiaries, the distribution of EC-funds and the decision making-process such as those concerning the work programme, the allocation of specific managerial responsibilities within the work plan, the structure and content of deliverables, budgetary issues, contract termination, or addition of partners. Those decisions will be proposed by the Coordinator and Management Support Team and validated in General Assembly. Decisions regarding the day-to-day research of the individual work packages will be made by the work package partners, but within the framework of the project guidance.

The Consortium Agreement further defines the rules for communication (internal, external). Within the Consortium Agreement the mechanisms for quality, risk, and conflict-management will be determined and additionally the IPR will be arranged (*e.g.* the access rights of background and exploitation of results, joint ownership agreement even after the end of the funded project, defaulting partner).

#### Internal communication and reporting structure

The General Assembly will be meeting annually to discuss the progress of the project and solve potential problems that have arisen. The content of all meetings will be recorded and circulated to the consortium and EC as a formal written record. Besides the regular meetings, the consortium will communicate for scientific/technical and administrative/financial aspects via telephone, video conferences, bilateral meetings, ad hoc meetings, and via the internal website.

The project follows the Horizon 2020 guidelines<sup>26</sup> for project reporting. With assistance of the Management Support Team, the Coordinator is responsible for administrative and financial planning and reporting. If the Coordinator or the Management Support Team recognise significant deviations in the financial planning, the EC will be informed in due time. The Steering Committee is responsible for scientific/technical as well as administrative/financial planning and reporting.

#### Conflict resolution

In order to mitigate most efficiently the risk of conflicts within the consortium, the following problem resolution strategies have been integrated into the management structures and work plans:

- **Handling a non-performing partner:** The work package leader must report to the Steering Committee in case a non-performing partner is observed. In the next step, the Steering Committee will communicate with the non-performing partner and address the situation. After establishing the problem, the Steering Committee will request an improvement in performance through corrective actions. This request will be provided in written form and within a reasonable timeframe.
- **Disputes of a scientific/innovative nature:** Given a scientific or innovation-based conflict arises and cannot be resolved after consultation with the involved partners, the responsible work package leader is to prepare a descriptive document in which the conflict's nature is precisely analysed. This report will be forwarded to the General Assembly, where a solution will be discussed and then voted on.
- **Intellectual Property Conflict:** Should conflicts on IP claims arise among the project partners, the Innovation Manager is to mediate between the conflicting parties. If this is not successful, the Innovation Manager will suggest a split between the partners, based on their input. The final approval of the Innovation Manager's proposal lies within the responsibility of the General Assembly.

<sup>25</sup> <http://www.desca-2020.eu/>

<sup>26</sup> [http://ec.europa.eu/research/participants/docs/h2020-funding-guide/grants/grant-management/reports/periodic-reports\\_en.htm](http://ec.europa.eu/research/participants/docs/h2020-funding-guide/grants/grant-management/reports/periodic-reports_en.htm)

- ❏ **Unlikely case of conflicts remaining unsettled:** A meeting with the concerned conflict party or parties, the corresponding WP leaders and the project coordinator will be convened. To avoid biased discussions, an independent person, if necessary externally appointed, will be responsible for chairing the meeting. Furthermore, this person will solve the conflict by suggesting and upon agreement, implementing an amicable solution.

### 3.2.3. Risk Management

In order to ensure a successful project implementation irrespective of unforeseen circumstances, BioICEP includes a risk management strategy. The Steering Committee will be responsible for monitoring the risks and will update the General Assembly in every meeting. The General Assembly can decide to adapt the risk strategy to adapt it to the changing circumstances during the progress of the project. The Steering Committee will take action when required to mitigate any risks, according to the plan. When necessary, follow-up meetings will be held by the Steering Committee with the participation of all relevant Partners. If risks occur that had not been identified and there is no predefined mitigation action, the Steering Committee will agree on the appropriate mitigation measures. Table 3.2 b gives the overview of the risks identified at this stage. For each specified risk appropriate actions are proposed in case the risk will occur.

#### Risk and contingency management

BioICEP is an ambitious project with a logical budget and multinational partnership. It consequently carries a medium level of risk that will need managing effectively. The risk management roles in the project are split into two: the Technical Manager and the activity supervisors will handle technical risk management while the Project Manager will manage all other risks. Before each stage of the project commences the appropriate manager will complete a full risk assessment. This will follow the process identification, evaluation and response planning. Prevention or reduction plans can be put in place of the risk can be accepted and tolerated. Alternatively, a contingency plan could be put into place and contingency trigger assigned. The impact of the risk will then be assessed and contingency resources planned. The consortium has already identified the basic risk and has drawn contingency plans, as presented in the relevant paragraph.

#### Critical risks

All technologies to be employed in this project are led by well experienced partners. Moreover, they are all well introduced in collaborative research and development work in international project. However, risk avoidance is also obtained by the potential overlapping of the partners in their competencies. This way every problem faced by one of the partners will obtain direct collaboration and help by another. Finally, a strict following of the timetable and the correct management of the project will be sufficient to achieve the desired objectives. The Risk Table below itemizes the technical risks in the *BioICEP* project.

#### Elaboration on each of the mitigation measures applied to the identified risks were identified.

Further information on the mitigation measures applying to each of the identified risks has been added to the Milestone Risk-mitigation Measures table and the critical risks for implementation below.

A risk management strategy is provided within the project in order to ensure a successful implementation of the objectives and delivery of stated impacts irrespective of unforeseen circumstances,. The General Assembly can decide to adapt the risk strategy to changing circumstances during the progress of the project in the interest in achieving optimal project outputs. The Steering Committee will take action when required to mitigate any risks, according to the plan. When necessary, follow-up meetings will be held by the Steering Committee with the participation of all relevant Partners. If risks occur that have not been identified and there is no predefined mitigation action in place, the Steering Committee will agree on the appropriate mitigation measures.

Risk Description	Risk Level	Proposed Risk-mitigation Measures	WP
Delays of key deliverables	Medium	The steering committee will closely monitor the timeline for key deliverable achievement. The risk management strategy will include a process where tasks involved in contributing to each deliverable are managed and those which are found to pose additional technical difficulties are examined with alternative technical approaches established to overcome any obstacles facilitating meeting the planned timeline	1



		In the event the deadline cannot be met, a provisional draft will be developed allowing any interdependent activities to be carried out. Milestones are placed to proactively control part of the work program where inter-dependencies may become critical and dedicated risk management strategies are foreseen for specific critical milestones.	743 - 01/10/2019
IPR or other conflict amongst the partners.	Low	<p>AIT has a strong IPR policy which will facilitate the smooth operation of IPR activities within the project. AIT is experienced in the management of multi partner academic and commercial projects involving proprietary technologies and products.</p> <p>The frequent communication foreseen in task 1.2 will allow early detection of potential issues. The coordinator will intervene to facilitate dialogue between involved partners, at the highest level. As a last resort, the conflict resolution measures clearly assigned and agreed upon in the consortium agreement will be activated.</p> <p>This includes mediation by the Innovation Manager between the conflicting parties. If this is not successful, the Innovation Manager will suggest a split between the partners, based on their input. The final approval of the Innovation Manager's proposal lies within the responsibility of the General Assembly.</p>	8
Management issues due to Cross continental consortium	Medium	<p>AIT has a dedicated EU-China engagement officer who has been instrumental in developing the strong relationship between the Chinese partners within the consortium. Hence this relationship will be further fostered throughout the project. The proposed working groups that will be chaired by the Technical Manager, have relevant representatives of the most relevant disciplines in order to assure the required leadership.</p> <p>AIT plans to travel to China early in timeline of the project in order to facilitate good cross continental appreciation and of understanding differences and similarities. The co-ordinator plans to use this knowledge to foster synergistic and beneficial outputs by marrying the different but complementary approaches of China and Europe. The highly positive and enthusiastic relationship already forged within this project to date bodes well for good cross continental management with a dynamic approach to overcoming any issues enabled by both European and Chinese partners.</p>	1
Financial risk.	Low	<p>AIT has a strong administrative and financial department experienced in H2020 project operation which will be beneficial in pre-empting and resolving any financial risks which can arise. The AIT administrative and financial department as strong policies regarding financial operation eg reading subcontracting, which are completely adherent with EC policies in these areas.</p> <p>Administrative/financial management will maintain a close financial monitoring process so as to constantly assess financial progress and be able to identify early signs of concern.</p>	1

The Technical Manager and the activity supervisors will be responsible technical risk management. Before each stage of the project commences a full risk assessment will be completed. This will follow the process identification, evaluation and response planning. Prevention or reduction plans can be put in place as necessary in order that risks can be declared acceptable. Alternatively, a contingency plan involving assessment of the impact of the risk and planning of contingency resources. Further information on the identified risks and contingency plans are provided in green writing below.

Moreover, they are all well introduced in collaborative research and development work in international project. However, risk avoidance is also obtained by the potential overlapping of the partners in their competencies. This way every problem faced by one of the partners will obtain direct collaboration and help by another. Finally, a strict following of the timetable and the correct management of the project will be

sufficient to achieve the desired objectives. The Risk Table below itemizes the technical risks in the *BioICEP* project.

**Table 3.2.b. Critical risks for implementation**

Risk Description:	RISK Level	Mitigation Measures:	WP
The MW reduction is not sufficient for enzymatic or microbial degradation	Low	<p>A number of different multifaceted approaches will be investigated to avoid reliance on a single method. Failsafe methods, while planned technologies are being upgraded, include incubating plastics such as plastic films/microplastic collected from the environment in saline and exposing to UV and accelerated aging.</p> <p>Furthermore the proprietary TCD sonic-green chemical technology has already shown promising results in achieving MW reduction of a range of recalcitrant plastics. This technology will be supplemented with additional methods during this project. Furthermore AIT will also develop innovative combination technology methods based on its considerable polymer engineering experience and facilities, which will be targeted at effective recalcitrant plastic MW weight reduction.</p>	2
Inadequate enzymatic cocktail degradation efficiency	Medium	<p>Multiple cutting edge technology approaches including new biosensors for accelerated discovery will alleviate these risks.</p> <p>The plan to simultaneously develop a series of different novel approaches will provide strong failsafe measures to ensure the sufficient enzymatic degradation will be achieved. These different measures include:</p> <ul style="list-style-type: none"> <li>- Immobilisation of enzymes for increased stability, reusability and cost reduction.</li> <li>- Whole-cell approaches &amp; microbial cell surface display</li> <li>- Screening of pure enzymatic activities for their potential to degrade plastics including the establishment of novel assays for screening plastics degradation activities (M24)</li> <li>- New accelerated screening by novel in situ biosensors which flag high performing strains</li> <li>- Improved biocatalyst activity and performance through directed evolution</li> </ul>	3
Microbial Consortia inhibited by factors such as contaminants	Medium	<p>The investigated range of defined species, cultivation conditions and adaptation protocols will be expanded to circumvent performance of the microbial consortia.</p> <p>During the pre-treatment processes chemicals may be released or formed that negatively impact microbial and/or enzymatic performance. An example of this the release of antimony trioxide, a catalysis used for polymerisation, from PET which is present in trace amounts. Metal tolerant and strains capable of dealing with possible inhibitory factors released during pretreatments of mixed plastic waste will be included in the microbial consortia. Plastics from the different pre-treatment methods will be used to test their impact on the degrading community stability and its ability to degrade the plastics. This process will be iterative and combined with the work from WP2 to select optimal pre-treatment processes that both make the plastic available for microbial breakdown but have limited impact on the microbial community or enzyme activity</p>	5
Bioproduct quality is insufficient for industrial applications	Medium	<p>The reasons, leading to the insufficient quality, will be indicated and feedback into previous WP establish measures for improvement will be implemented. Metabolic models will be upgraded to improve bioprocessing and bioproduct production. Bioproduct blends will be developed to enhance properties to levels required.</p>	6



Risk Description:	RISK Level	Mitigation Measures:	WP
		<p>Partners including AIT and TCD are highly experienced in the compounding and the development of blended and composite plastics for industrial applications. Combination on the bioplastic products with other biodegradable plastics and additives will be carried out as required.</p> <p>In addition, alternative high performance bioplastics such as Polyethylene furanoate and its derivative which are highlighted as a promising sustainable alternative to its ubiquitous and market dominant petroleum-based counterpart polyethylene terephthalate (PET).</p>	<p>Associated with document Ref. Ares(2019)6080743 - 01/10/2019</p>
Integrated operation of the pilot plant is problematic.	medium	<p>A modular pilot plant will be initially developed to enable bottlenecks to be bypassed, while modified technology solutions are developed to overcome any obstacles within any of the individual processes.</p> <p>The reactor setup will consist of three operation units: (i) biocatalytic degradation of the pretreated plastics using enzymatic cocktails followed by consortium of strains, (ii) separation of the biomass and residual plastic components from the nutrient-rich fluid (using disk centrifugation), and (iii) microbial PHB/rhamnolipid/nanocellulose production using the nutrient-rich effluent stream of the first reactor as influent. These units will enable independent operation allowing any problematic operation to be isolated and resolved.</p> <p>A series of options and parameter adjustments are available to trouble shoot the pilot plant operation including:</p> <ul style="list-style-type: none"> <li>- Specifications for pilot reactor setup including important reactor parameters such as the optimum pH, temperature, aeration (strict aerobic, micro-aerobic or strict anaerobic), agitation, solid residence time (SRL), hydraulic residence time will be provided from previous WP, in particular WP6 and will be optimised as part of WP 7 tasks.</li> <li>- Options for continuous or batch-wise feeding</li> <li>- Harvesting options: continuous effluent efflux, batch wise efflux of homogeneous effluent or batch wise sedimentation and efflux of fluid effluent</li> <li>- Modular operation allowing individual regulation of biocatalytic and microbial pretreated plastics degradation processes in accordance with the parameters developed in WP</li> <li>- Feedback of the bioproduct analysis and processing performance characteristics will be used to optimise the production process in conjunction with consortium partners</li> </ul> <p>The modular design of the pilot plant, combined with selective options and operation parameter optimisation will enable effective mitigation of any operational issues.</p>	7

The Milestone Risks are managed by the Project Manager and monitored by the Steering Committee with updates provided to the General Assembly. For each identified risk, additional details on the appropriate actions proposed in case of risk occurrence is provided in green writing.








































































































### 3.3. Consortium as a whole

The BioICEP pan European-Chinese consortium has been assembled to meet the specific needs of the project combining polymer processing and industry specialists with microbiology, enzymology, molecular geneticists, and bioprocessing experts. This team of academic and industry focused research partners is well balanced and fully equipped to efficiently and effectively deliver the project objectives and ensure their exploitation. The **nine country two continent** geographical span of the consortium is completely appropriate to the

environmental plastic challenge addressed here by the BioICEP project. The contribution of key innovative technologies by both Chinese and EU partners are the deliverables of the ambitious BioICEP technology.

The consortium combined diverse and specific capacities and the roles of each partner, identified as optimal to meet the project objectives are outlined in the table that follows.

Table 36 - Capacities and roles of the consortium participants

	Abbreviation	Nationality	Status	Plastics Processing	Plastics Recycling	Bioplastics	Microbiology Engineering	Biocatalysis	Bioprocessing	Industry Clientele	Industry Supports	Dissemination/Communication	Exploitation and Technology Transfer	Administration
 <b>AIT</b> Instituto Tecnológico de Aithios Athlone Institute of Technology	AIT	IE	RPO											
 <b>acteco</b> Buenos por naturaleza	ACT	ES	SME											
 <b>AIMPLAS</b> INSTITUTO TECNOLÓGICO DEL PLÁSTICO	AIM	ES	RPO											
 <b>Avecom</b> Bioproducts & Apps	AVE	BE	SME											
 <b>TU Clausthal</b> Clausthal University of Technology	CUT	DE	RPO											
 <b>IMITH</b> IMGGGE	IMGE	RS	RPO											
 <b>iBET</b> Instituto de Biología Experimental e Tecnológica	iBET	PT	RTO											
 <b>LIT</b> LIMERICK INSTITUTE OF TECHNOLOGY	LIT	IE	RPO											
 <b>Logoplaste</b> SHAPING THE FUTURE TOGETHER	LOG	PT	SME											
 <b>microlife solutions</b>	MLS	NL	SME											
 <b>National Technical University of Athens</b>	NTUA	EL	RPO											
 <b>Trinity College Dublin</b> Coláiste na Tríonóide, Baile Átha Cliath The University of Dublin	TCD	IE	RPO											
 <b>Beijing Institute of Technology</b>	BIT	CN	RPO											
 <b>山东大学</b> SHANDONG UNIVERSITY	SDU	CN	RPO											
 <b>中国科学院微生物研究所</b> Institute of Microbiology, Chinese Academy of Sciences	CAS	CN	RPO											

**Proven collaboration:** A majority of the partners have worked before in European projects and all of have extensive experience in international cooperation within fields related to the ecological impact of plastics. Many of the partners have worked together in various projects having established relationships and cooperation structures. As shown above the partners are complementary in various levels and match the project's objectives. BioICEP internships / secondments will to be offered exclusively to consortium members, serving to enhance balanced EU-China collaboration within the project.

Over 50 % of EU partners are industry, industrial liaison focused, (ACT, AIM, AIT, iBET, LOG, and MSL), which is valuable in supporting the transition of the technology from TRL3 to TRL5, and ensuring that there is a clear route to the exploitation of the results. This is consistent with the draft PDER presented in Section 2.2, where partner involvement in the development of the technology to high TRL post-project is foreseen.

### 3.4. Resources to be committed

The consortium set up in this project has clear vision of its objective and therefore is committed to the appropriate allocation of the financial resources at its disposal. The close nature of cooperation in the project is evident by the coherent program that has a joint focus while distributing tasks among the different partners that have complementary expertise and resources. The budget distribution is described in details in Table 40. The project financial plan has been designed to cover the partners' needs for the accomplishment of the project targets. A balanced fund distribution has been made. In the following the distribution of Person Months per partner per work package is illustrated.

Table 37: 3.4b Other direct cost items (travel, equipment, other goods and services, large research infrastructure)

1 AIT	Cost (€)	Justification
Travel	39,200	Travel (32 trips, - 2 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
Equipment		
Other goods & services	95,410	Materials, reagents and consumables for waste treatment and characterization of materials. Dissemination events and activities, Conferences and meetings and communication activities and Business model preparation. Audit certificate.
Total	134,610	
2 ACT	Cost (€)	Justification
Travel	22,400	Travel (9 trips, 2 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project at an average cost of € 800 pp.
Equipment	0	
Other goods and services	7,600	Materials and consumables for waste treatment. Dissemination activities, Conferences and meetings and communication activities
Total	30,000	
3 AIM	Cost (€)	Justification
Travel	28,800	Travel (19 trips, 1-3 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
Equipment	0	
Other goods & services	66,400	Catalyzers, solvents, raw materials, gases, extrusion auxiliary tooling, reactivities, MW susceptors, MW vessels and accessories. Dissemination events and activities, Conferences and meetings and communication activities. Website and video development and distribution Audit certificate
Total	95,200	
4 AVE	Cost (€)	Justification
Travel	7,200	Travel (7 trips, 1-2 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
Equipment	5,000	Pilot plant reactors and pumps
Other goods & services	80,000	Pilot plant programming. Fermentation, separation and degradation consumables, lab analysis. Dissemination events and activities, Conferences and meetings and communication activities. Audit certificate
Total	92,200	
5 CUT	Cost (€)	Justification
Travel	16,000	Travel (13 trips, 1-2 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
Equipment		
Other goods & services	30,200	Consumables, lab analysis, Conferences and meetings and communication activities
Total	46,200	
6 IMGGE	Cost (€)	Justification
Travel	12,200	Travel (10 trips, 1-2 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
Equipment	53,000	Bio-reactors, chemical reactor, plate reader spectrometer
Other goods & services	87,000	Consumables for directed evolution and microbial and enzymatic screening purposes Dissemination events and activities, Conferences and meetings and communication activities. Audit certificate
Total	152,800	
7 iBET	Cost (€)	Justification
Travel	19,200	Travel (15 trips, 1-3 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
Equipment	105,500	Bio-reactors, gas standards and reaction monitoring sensors
Other goods & services	13,000	Accessories, consumables, lab analysis, Matlab software fees. Dissemination events and activities, Conferences and meetings and communication activities. Audit certificate
Total	137,700	
8 LIT	Cost (€)	Justification

<b>Travel</b>	14,400	Travel (14 trips, 1-2 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
<b>Equipment</b>	0	
<b>Other goods &amp; services</b>	28,500	Consumable and reagents. Dissemination events and activities, Conferences and meetings and communication activities. Audit certificate
<b>Total</b>	42,900	
<b>9 LOG</b>	<b>Cost (€)</b>	<b>Justification</b>
<b>Travel</b>	15,200	Travel (15 trips, 1-3 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
<b>Equipment</b>	12,086	Polymer dryer
<b>Other goods &amp; services</b>	9,500	Extrusion Blow Molding materials and equipment customisation accessories. Dissemination events and activities, Conferences and meetings and communication activities.
<b>Total</b>	36,786	
<b>10 MLS</b>	<b>Cost (€)</b>	<b>Justification</b>
<b>Travel</b>	12,000	Travel (11 trips, 1-2 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
<b>Equipment</b>	30,500	FPLC (enzyme purification from white-rot fungal cultures, ) and computer
<b>Other goods &amp; services</b>	45,000	Laboratory Consumables. Dissemination events and activities, Conferences and meetings and communication activities. Audit certificate
<b>Total</b>	87,500	
<b>11 NTUA</b>	<b>Cost (€)</b>	<b>Justification</b>
<b>Travel</b>	27,200	Travel (17 trips, 2 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
<b>Equipment</b>	40,000	HPLC equipment for bioreagent analysis,
<b>Other goods &amp; services</b>	43,000	Consumables, reagents and labware. Dissemination events and activities, Conferences and meetings and communication activities. Audit certificate
<b>Total</b>	110,200	
<b>12 TCD</b>	<b>Cost (€)</b>	<b>Justification</b>
<b>Travel</b>	20,000	Travel (17 trips, 2 people), including to China, dissemination activities. Participation in, conferences and exhibitions during the 4 year project (average cost of € 800 pp.)
<b>Equipment</b>	15,000	Polymer processing equipment
<b>Other goods &amp; services</b>	41,745	Laboratory consumables, materials and reagents Audit certificate
<b>Total</b>	76,745	

## 4. Members of the Consortium

Associated with document Ref. Ares(2019)6080743 - 01/10/2019

### 4.1 Participants

The participants span *nine countries and two continents, Europe and China*. The EU coordinator is Dr. Margaret Brennan Fournet at AIT and the Chinese coordinator is Prof. Qingsheng Qi (email: [qiqingsheng@sdu.edu.cn](mailto:qiqingsheng@sdu.edu.cn)) at SDU China. The participants are listed in Table 38 and a description of each is provided in the following section.

Table 38: The Participants

	Abbrev	Nation	Status	Entity
	AIT	IE	RPO	Beneficiary
	ACT	ES	SME	Beneficiary
	AIM	ES	RPO	Beneficiary
	AVE	BE	SME	Beneficiary
	CUT	DE	RPO	Beneficiary
	IMGGE	RS	RPO	Beneficiary
	iBETT	PT	RTO	Beneficiary
	LIT	IE	RPO	Beneficiary
	LOG	PT	SME	Beneficiary
	MLS	NL	SME	Beneficiary
	NTUA	EL	RPO	Beneficiary
	TCD	IE	RPO	Beneficiary
	BIT	CN	RPO	International Partner
	SDU	CN	RPO	International Partner
	CAS	CN	RPO	International Partner



## Section 1. General Partner Information

1. Partner name: Athlone Institute of Technology
2. Partner Website: <https://www.ait.ie/>
3. Participant Identification Code (PIC) No: 996870747
4. Contact person name and email address:
  - a) Dr. Margaret Brennan Fournet ([mfournet@ait.ie](mailto:mfournet@ait.ie))
  - b) Dr. Declan Devine ([ddevine@ait.ie](mailto:ddevine@ait.ie))
5. Position in organization:
  - a) Senior Researcher
  - b) Institute Director
6. Department name: Materials Research Institute
7. Average Person Month Rate in the organization: 7500euro



## ACADEMIC PARTNER PROFILE

## Section 2. Technical Part Contribution

a) Which is the role of your organization in the proposal? *(Please explain in two sentences. Thank you.)*

Coordination and management of work packages related to plastics pre-treatment, microbial strains, enzymatic engineering, biopolymer fermentation, degradation & regeneration as well as dissemination and communication of work package outcomes.

b) Which is the current state of the art of the technology you will introduce/progress during the project? Please provide a review of the recent scientific papers in the domain, patents and projects according to your knowledge. Thank you.

BioICEP aims to introduce a technology that mimics and operates in tandem with nature using microbial and enzymatic digestion of recalcitrant and degradable plastic waste, funneling the resulting carbonaceous resources for the fermentation of new equivalent biopolymer plastics. In this manner, degraded polymer components such as monomers and oligomers will be recycled in a system that close follows the providence of nature and enables the regeneration of new readily biodegradable plastics, creating a wholly circular plastic life cycle. The development of the BioICEP technology will deliver a new completely sustainable solution, with the potential to strongly contribute to the clean-up of the world's plastic waste crisis. BioICEP can achieve high carbon efficiencies and is a pertinent technology for our environmentally secure future.

c) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

Disruptive Technologies Innovation Fund. INSPIRE - Innovative Sustainable Packaging Ireland Packaging a better tomorrow. The project (presently) aims to utilise biomass and food waste as feedstock for the synthesis of next-gen biopolymers to replace existing petroleum-based plastics for the fabrication of biodegradable and compostable packaging materials. Design disruptive technologies include the potential for enzyme modification of existing polymeric materials present in mushroom and other waste streams rich in natural polymeric materials by testing for antioxidant and antimicrobial properties generating useful added value by-products for incorporation into biodegradable packaging.

d) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

The current endeavour to complete the life cycle for plastic polymers, though ultimately the optimal route to resolving the world's plastic crisis is hampered due to a number of factors which we will attempt to resolve through coordination of the various work packages to be carried out by consortium partners:

- Obstacle: The natural evolution of microbes to degenerate new materials is slow. Microbes have a natural propensity to degenerate material and indeed to evolve to degrade new materials in the upkeep of nature's cycle of generation, degradation and regeneration. However this process is intrinsically slow. Waste plastic

substrates upon which microbes can act have only become prevalent in the past number of decades. The search for newly evolved plastics degrading strains is extensive and exhaustive, with only a limited number of strains discovered to date that have reasonable plastic degradation efficiencies.

- **Obstacle: Fundamental principles of microbial and enzymatic degradation of plastics are highly complex**  
Considerable and intensive scientific efforts are required to elucidate the complex underlying microbial and enzymatic degradation mechanisms, which are essential to the improvement of efficiencies and performance. These challenges are compounded by the fact that while individual strains may be operated to degrade a specific plastic, there are often strong inhibiting factors preventing communities of microbial and fungal strains functioning in tandem to degrade plastics and plastic mixtures.
- **Obstacle: Focus on single strains and single platforms operating on individual rather than mixed plastics**  
Approaches to date have primarily focused on the development of individual strains and enzyme sets rather than culturing mixed consortia and cocktails. Microbial communities or mixed cultures with defined microbial strains are needed to achieve efficient biodegradation of plastics such as PE, and PS which have a lack of hydrolysable functional groups in their backbones.<sup>1</sup> Compared with the use of single microorganisms, microbial communities and enzyme cocktails are essential to successful high efficiency biodegradation of plastic mixes.
- **Obstacle: High carbon footprint of other approaches**  
Approaches to date often include high carbon foot print processes to prepare plastics for microbial and enzymatic attack
- **Obstacle: Lack of a concerted industry focused approach**  
Approaches to date are often carried out in isolation without appropriate consideration of the multifaceted factors that need to be addressed such as capacity for reduction to practise, economic suitability for industry buy in and competitively with in the market.

e) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- Project management
- General assembly meeting and consortium communication
- Administrative and financial management
- Establishment of novel assays for screening microbial enzymes for plastics degradation potential (plate and liquid assays using model compounds and their defined mixtures) and surface degradation analysis
- Quantitative/qualitative analysis of plastic breakdown potential and dynamics
- Development and optimization of downstream procedures for products' recovery
- Pilot reactor set up
- Extensive chemical, mechanical, thermal and aging characterization and analysis
- Demonstration of pilot production of PHB and nanocellulose for thin biopolymer film production for applications such as food packaging and rhamnolipids for pharmaceutical products.
- Life cycle analysis of BioICEP technology and products
- Business model development

f) Which are the deliverables that your organization will deliver during the project

- Internet based communication platform and repository
- A report with the minutes of all meetings
- A report with all video conference meeting of the steering committee
- Report on the screening of pure enzymatic activities for their potential to degrade plastics
- Establishment of communities with high resilience to the contaminants and chemicals present in pre-treated plastics and mixed plastics.
- Quantitative/qualitative analysis of plastic breakdown potential and dynamics
- Protocols and report on the optimized conditions for downstream process
- Operation of modular integrated BioICEP pilot scale plant demonstrating the biocatalytic and microbial breakdown of 20%+ of mixed plastics.
- Small scale pilot production of high performance PHB and nanocellulose for applications such as food packaging and rhamnolipids for pharmaceutical applications.
- Report on life cycle analysis study demonstrating the low environmental impact of BioICEP and its favorable position compared with current and emergent competitor technologies.



- Business model presenting the go to market potential and market projections for BioICEP.
- g) Which are the technical objectives that your organization needs to achieve during the project?

As project coordinator and manager we must ensure the following objectives are achieved during this project:

- All green solution using only mechanical and optical pre-treatments which make plastics amenable to microbial degradation
  - Accelerated screening (Served by novel in situ indicator biosensors)
  - Targeted collection and screening for the most plastic potent strains (Served by accessing the most polluted sites where global sites where microbe have had the longest and most intensive opportunities to evolve Accelerated and directed evolution (Served by novel CRISPR-9cas technology and
  - Augmented strain activity using a number of innovative bioengineering approaches. (Served by metabolic engineering to incorporate multiple pathways into a single strain platform ...)
  - Enhanced plastics degradation propensity (Served by thermally stabilised/cross linked enzymes blended within the plastics
  - Building of novel consortia with defined microbial strains and enzyme combinations that can operate in tandem for increased degradation performance compared to individual strains. (Served by synergy tests, sequencing of discovered novel strains, defined enzyme mixtures, defined co-cultured strains)
  - Market informed targeting of high demand biopolymer food packaging products based on improved performance biopolymers (Served by prioritising nanocellulose and PHB production with high performance mechanical and processing properties, over widely investigated lower performance PHAs).
  - Demonstrate the capacity for economic reduction to practice with a pilot scale plan. (Served by a plan for process and automated
  - Market lead project design, which directly answers market and societal needs. (Served by strong industrial engagement
  - Demonstration of low carbon foot print (Served by LCA benchmarking compared with other current approaches))
- Please provide info regarding the market of the technology that your organization will develop (*size of market, main competitors, costs of services/products etc.* )

## ACADEMIC PARTNER PROFILE

### 3. Partner Profile Information

#### a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

Athlone Institute of Technology (AIT) is an education and research institution located in Ireland and was named Institute of Technology of the Year 2018. AIT has an international reputation in polymer processing and is a key provider of graduates into the industry. AIT hosts the Enterprise Ireland funded Technology Gateway Centres for Polymer Processing (Applied Polymer Technologies) and Connected Media (COMAND). AIT are involved with 6 of the 16 SFI Centres - Confirm, Amber, Connect, Adapt, SSPC, CURAM. AIT is also one of the four RPOs of the Irish Composite Centre. AIT hosted the European Composites, Plastics and Polymer Processing Platform (ECP4) Annual meeting May 2018. AIT is delivering or has delivered +30 Innovation Partnerships, 8 Commercialisation Funds, +400 Innovation Vouchers and 19 Fusion Awards since 2000.

- AIT hosts 2 of the 15 Enterprise Ireland funded Technology Gateway Centres, which work closely with industry to deliver technology solutions for Irish industry close to their market needs. One of the TGP centres is Applied Polymer Technology (APT) Centre.
- AIT has involvement with 5 of the 16 SFI Centres (Confirm, Amber, Connect, SSPC, CURAM). AIT is also one of the four RPOs of the Irish Composite Centre (ICOMP).
- AIT hosted the European Composites, Plastics and Polymer Processing Platform (ECP4) Annual meeting May 2018.
- AIT is a partner in a Technology Transfer consortium with Waterford IT and IT Carlow and led by Maynooth University, funded by Enterprise Ireland under the Technology Transfer Strengthening Initiative.
- AIT holds the award from the EU Commission for HR Excellence in Research
- AIT was named Institute of Technology of the Year 2018. AITs strengths come from identifying areas of skills shortage and working with businesses to improve links between business and academia

b) Relevant elements of the Curriculum Vitae/Biography (including profile pictures) of the team responsible for the project implementation. (Please provide maximum 2 paragraphs per person and refer to the gender of each employee. Thank you)

#### Dr Margaret Brennan Fournet



Dr Margaret Brennan Fournet received her B.Sc. in Experimental Physics at University College Dublin, and her PhD in the field of Nanomaterial Nonlinear Optics at the School of Physics Trinity College Dublin. On returning from a visiting fellowship at the National Laser Centre at the Australian National University, Canberra, Margaret lectured and established an interdisciplinary research team at the National University of Ireland, Galway working on nanostructure plasmonics for applications in biomedical diagnostics and printed electronics. In 2010, Margaret was appointed as a lecturer at the School of Physical Sciences at Dublin City University. In 2012, Margaret took the position of Chief Technology Officer at PixinBio SAS, a spin out company from the Fresnel Institute as the CNRS, Marseille in France, developing all electronic diagnostic devices. Margaret was awarded a Marie Curie Fellowship at the Department of Bioelectronics at the Ecole Nationale Supérieure des Mines de St. Etienne, France, in 2013 where she carried out novel integration of nanophotonics with plastic bioelectronics.

In 2017, Margaret joined the Materials research institute at the Athlone Institute of Technology. Her research is centered on harnessing the power of nano-scale plasmonics for interfacing with biological systems. She has also focused on developing nano-active probes that mimic and facilitate operation in tandem with the physiological environment, allowing new in situ modalities for monitoring and stimulating biological systems

#### Dr Declan Devine



Dr Devine is the director of the Materials Research Institute (MRI) in AIT. Dr Devine holds a PhD in Biopolymer Engineering (2006) from AIT, where he also completed undergraduate studies in polymer engineering. Following his PhD studies he worked on an industry based post-doctoral fellowship which enabled the development, patenting and licensing of orientated polymeric films for use in the ophthalmic industry to Transitions Optical Ltd. He subsequently gained international research experience as a Senior Project Leader in the Preclinical Services Programme at the AO Research Institute in Switzerland, Institute where he was responsible for managing preclinical studies for a variety of industrial and academic collaborators. Dr Devine was awarded a Marie Curie Fellowship in 2012 and was named an Irish champion of EU research (Sept 2012) and the Marie Curie Fellow of the

week (Oct 2014). This work enabled Dr Devine to receive training in world leading research centres namely; Harvard Medical Schools Center for Advanced Orthopaedic Studies and the Mayo Clinic's Rehabilitation Medicine Center in the field of bone tissue engineering.

Dr Devine's current research interests centres on the development of materials for biomedical applications such as bone regenerations and biodegradable polymer stents, and structural thermoplastic composites. Dr Devine has published in the fields of controlled release from medical device coatings, 3D printing scaffolds and bone regeneration. He also currently supervises students in the area of material formulations and biodegradable stents. Dr Devine maintains several collaborations in these fields across the EU and at international centres located in the USA, Canada, Brazil and Switzerland.

#### Dr Yuanyuan Chen

Dr Yuanyuan Chen received her honour degree in Nursing from Southwest Medical University, Sichuan, China and have three



years' work experience as a staff nurse in Emergency Room, cardiology department, rheumatology department and department of diabetes, endocrinology and metabolism in Sichuan Provincial Hospital. She also received an honour degree in Mechanical Engineering and PhD in the field of 3D printing biodegradable coronary stents from Athlone Institute of Technology, Ireland. She has won the "Woman in Research" award in 2018 and is currently working as a postdoc researcher in Material Research Institute, Ireland. She has also won several EU COST action training funding and was trained in antimicrobial testing techniques by Academic Medical Centre, Amsterdam University, Netherland, numerical modelling in ureteric stents by Mathematic Institute, University of Oxford, England, patient-specific bone tissue engineering in Vienna University of Technology,

Austria.

#### Research Interests:

1. Biodegradable polymer composites for medical applications
2. Antimicrobial polymer composites

3. 3D printing patient-specific medical implants
4. Plastic packaging and recycling

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c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

1. Cormac McGarrigle, Ian Rodgers, Alistair McIlhagger, Eileen Harkin-Jones, Ian Major, Declan Devine, Edward Archer (2017). Extruded Monofilament and Multifilament Thermoplastic Stitching Yarns/Fibers, In Press uncorrected proof
2. Z Cao, M Daly, LM Geever, I Major, CL Higginbotham, DM Devine (2016). Synthesis and characterization of high density polyethylene / peat ash composites Composites Part B, 94: 312-21
3. YY Chen, LM Geever, JA Killion, JG Lyons, CL Higginbotham, DM Devine (2016). A Review of Multifarious Applications of Poly (Lactic Acid). Polymer-Plastics Tech Eng. 55(10):1057–1075.
4. Noel M. Gately and James E. Kennedy (2017). The Development of a Melt-Extruded Shellac Carrier for the Targeted Delivery of Probiotics to the Colon Journal of Pharmaceutics doi:10.3390/pharmaceutics9040038
5. Z Cao, M Daly, L Clémence, LM Geever, I Major, CL Higginbotham, DM Devine (2016). Chemical Surface Modification of Calcium Carbonate Particles with stearic acid using different treating methods Applied Surface Science, 378:320-329

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

1. European Society of Sonochemistry Meeting, 2020
2. Symposium on Biotechnology for Fuels and Chemicals 2021.

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

1. Polymer processing equipment: Twin screw compounding (Leistritz 27 mm, APV 19mm, Two Prism 16mm), Betol Single Screw extruder, Boston Matthews Single Screw Extruder, Supercritical fluid assisted extrusion.

2. Analytical equipment: High pressure liquid chromatography HPLC, Gas chromatography mass spectroscopy GC-MS, Fourier transform infraRed FTIR spectroscopy, Gel Permeation Chromatography GPC, Karl Fishcher Coulometer, Differential scanning calorimetry DSC, Thermogravimetric analysis TGA, Dynamic mechanical thermal analysis DMTA, Scanning electron microscope system, Mechanical properties analysis, Product shelf life testing, Goniometer, Rheometry, X-Ray diffraction,



## Section 1. General Partner Information

1. Partner name: ACTECO PRODUCTOS Y SRVICIOS S.L.
2. Partner Website: acteco.es
3. Participant Identification Code (PIC) No: 951168712
4. Contact person name and email address: Angel Martinez-Leon
5. Position in organization: Recycling Director
6. Department name: Recycling
7. Average Person Month Rate in the organization:

Logo of the  
organization



## INDUSTRY PARTNER PROFILE

## Section 2. Technical Part Contribution

- a) Which is the role of your organization in the proposal? *(Explain in 2 sentences. Thank you.)*

Acteco participate in Action 1. Mixed plastics pre-treatment, could be collaborate in Action 4 Recovery of carbonaceous components for biopolymer fermentation and Action 5 High yield fermentation of highli processble polymers, Action 6 Pilot scale integrated biopolimer fermentation from waste plastic and of course in Action 7 Dissemination and explotaiton

- b) Which is the current state of the art of the technology you will introduce/progress during the project? *Please provide an overview of commercial solutions and initiatives in scientific community in the domain, patents and projects according to your knowledge*

Polymer sorting and processing technology from our company will be introduced to this project. A combination of mixed plastic wastes will be designed to promote the optimization of microbial degradation. We will assist and provide polymer wastes.

- c) Does your team have recent relevant scientific papers in this domain? (no more than 4 years old) Please provide a full list

- N/A.....

- d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

- N/A.....e
- 

- e) Does your company provide related services or products as the ones mentioned in the project? *Please provide more information regarding the content and the clientele receiving this kind of services/products. Thank you.*

- We provide comprehensive environmental consulting and advice services
- We recycle and pellet plastics, including ABS, polypropylene, polyethylene, polystyrene.
- We also collect and transport waste from our customers
- We also supply waste optimisation equipment, such as container, compacters, bailers, industrial parts cleaning machines, rotocompactors and waste cages.

- f) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- Preparation of industry informed mixed waste plastic stocks
- Development of a novel combination of mechano-biochemical processes for the reduction of mixed plastic polymer molecular weight by 25-50%
- Develop a strong dissemination and communication campaign

h) Which are the deliverables that your organization will deliver during the project?

- Development of bioprocesses for the production of PHB with distinct monomer composition and functional properties, using waste synthetic plastics' monomers as feedback. Communication
- Mixed waste plastic stocks prepared

i) Which are the technical objectives that your organization needs to achieve during the project?

To develop bioprocesses for the production of PHB with distinct monomer composition and functional properties, using waste synthetic plastics' monomers as feedback.

To prepare mixed waste plastic stocks

j) Please provide info regarding the market of the technology that your organization will develop. Thank you. *(size of market, main competitors, costs of services/products etc.)*

## INDUSTRY PARTNER PROFILE

### 3. Partner Profile Information

a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

Acteco is an environmental company with big experience in collect and treatment all kind of waste, plastic, food waste, oil, carboard, (350.000 ton/year) hazardous waste (44.00 ton/year), contaminate water, ..... and we produce 12.000 ton/year of recycling plastic .....

b) Relevant elements of the Curriculum Vitae/Biography *(including profile pictures)* of the team responsible for the project implementation *(Please provide maximum two paragraphs per person and refer to the gender of each employee. Thank you.)*

Angel Martinez Leon - Aeronautical Engineer Research in more than 10 different R&D project

Luis Gonzalez – Agricultural Engineer Research in 4 different R&D project

Francisco Colomina. Chemical Degree Reserch in 4 different R&D project

Nuria Llopis. Chemical Degree Reserch in 3 R&D project. Expertise in Food contact industry

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

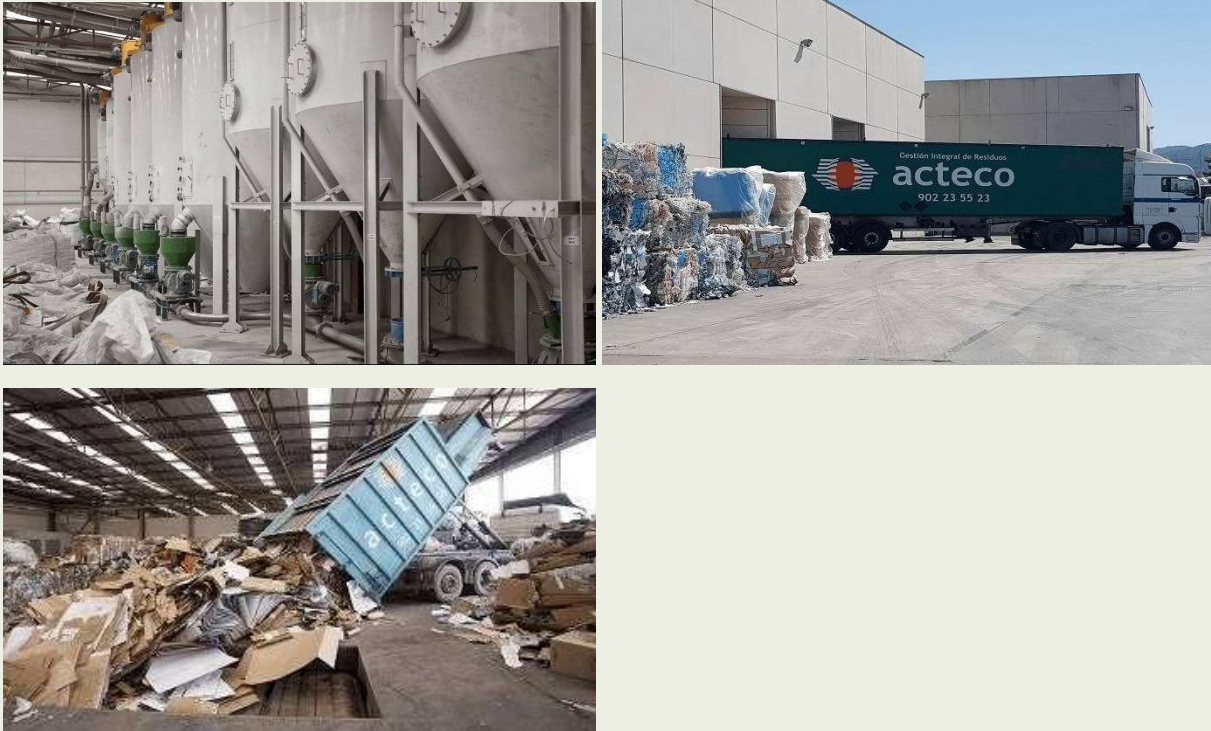
1. N/A.....  
.....



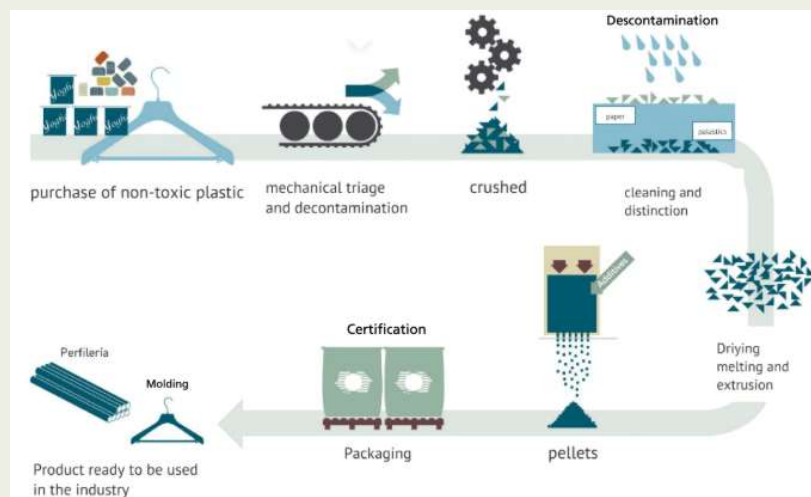
d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you. Associated with document Ref. Ares(2019)6080743 - 01/10/2019

1. Empresas que cambian el mundo Congreso de los Diputados Madrid 2018
2. Suchem Congres Universidad de Zaragoza 2017.
3. 1st INTERNATIONAL RECYCLING FORUM Agricultural for Plastics- Potential Recycling Wiesbadem 2015

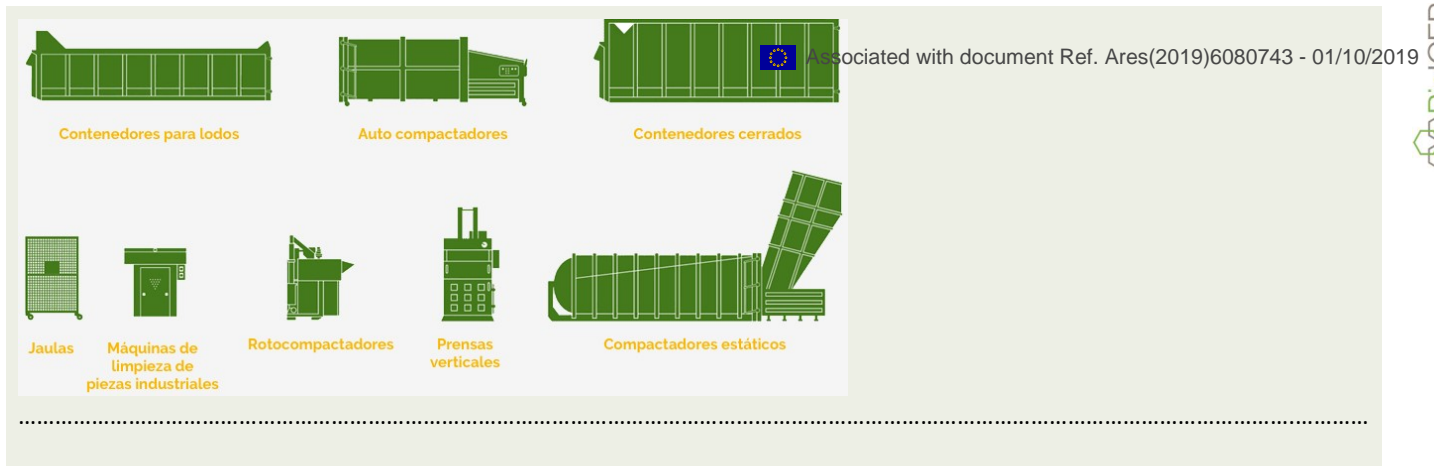
e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.



Our recycling process



Equipment relevant to this project:



## ACADEMIC PARTNER PROFILE

### Section 1. General Partner Information

1. Partner name: ASOCIACION DE INVESTIGACION DE MATERIALES PLASTICOS Y CONEXAS (AIMPLAS)
2. Partner Website: [www.aimplas.net](http://www.aimplas.net)
3. Participant Identification Code (PIC) No: 999513415
4. Contact person name and email address: Concha Sanz [proyectos@aimplas.es](mailto:proyectos@aimplas.es)
5. Position in organization: Technical deputy director
6. Department name:
7. Average Person Month Rate in the organization: 3504



## ACADEMIC PARTNER PROFILE

### Section 2. Technical Part Contribution

a) Which is the role of your organization in the proposal? *(Please explain in two sentences. Thank you.)*

AIMPLAS is the leader of WP of Dissemination, Exploitation and Communication. AIMPLAS will participate in WP2, focused on the pretreatment to generate substances for microbial treatment.

b) Which is the current state of the art of the technology you will introduce/progress during the project? Please provide a review of the recent scientific papers in the domain, patents and projects according to your knowledge. Thank you.

Based on the final structure of WP2, we will provide the current state of the art of the technology

c) Does your team have recent scientific papers in this domain? (no more than 4 years) (Please provide a full list. Thank you)

- VIDAL R, MOLINER E, MARTIN PP, FITA S, WONNEBERGER M, VERDEJO E, VANFLETEREN F, LAPENA N, GONZALEZ A: Life Cycle Assessment of Novel Aircraft Interior Panels Made From Renewable or Recyclable Polymers With Natural Fiber Reinforcements and Non-Halogenated Flame Retardants. *Journal of Industrial Ecology* 2018, 22:132-144.
- KACHRIMANIDOU V, KOPSAHELIS N, VLYSIDIS A, PAPANIKOLAOU S, KOOKOS IK, MARTINEZ BM, RONDAN MCE, KOUTINAS AA: Downstream Separation of Poly(Hydroxyalkanoates) Using Crude Enzyme Consortia Produced Via Solid State Fermentation Integrated in a Biorefinery Concept. *Food and Bioproducts Processing* 2016, 100:323-334. Notes: Part Number: A
- Waste and Waste Management. Chapter Municipal and Industrial Waste: Sources, Management Practices and Future Challenges. Brenda Bryant, Betty Hall (Editors). April 2018. Authors: Giulio Malucelli, Belén Monje. ISBN: 978-1-53613-441-4



d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

- LIFE EXTRUCLEAN Demonstrative Project for the removal of hazardous substances during the recycling process of polyethylene hazardous waste packages, employing supercritical carbon dioxide(sc-CO<sub>2</sub>): (LIFE13 ENV/ES/000067) scCO<sub>2</sub> system to be applied on recycled materials to be decontaminated
- CLIPP+: Manufacture and commercialization of high-quality recycled polyolefin films using an innovative H2020 GA N° 673663. scCO<sub>2</sub> system to be applied on recycled materials to be decontaminated
- KARMA2020 Industrial feather waste valorisation for sustainable keratin-based materials. H2020, GA N°723268. Waste valorisation to obtain biopolymers
- DAFIA: Biomacromolecules from municipal solid bio-waste fractions and fish waste for high added value applications.H2020 GA 720770. Bioplastics and high-value additives from MSW and marine rest raw materials.
- URBANREC New approaches for the valorisation of URBAN bulky waste into high added value RECYCled products. H2020, GA N° 690103. Valorisation of waste into high-value recycled products.
- ENZOX2: New enzymatic oxidation/oxyfunctionalization technologies for added value bio-based products. H2020 GA 720297. Application of enzymes and biotechnology to develop bioplastics and high added value products.

e) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

We will try to address the challenge of obtaining suitable high content of monomeric compounds from individual plastics, bio-based polymers and mixed plastic waste using depolymerization pre-treatments

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- Task 2.2 Pyrolysis: Subtask 2.1 thermal degradation of plastics assisted by MW
- Task 2.3 Reactive extrusion: Subtask 2.3.1. Controlled degradation by REX of individual plastics & Subtask 2.3.2. Degradation of mixed plastic waste
- Task 2.4 Depolymerization and thermodegradation assisted with scCO<sub>2</sub>: Subtask 2.4.1 Depolymerization treatments assisted with scCO<sub>2</sub> of individual plastics & Subtask 2.4.2 Depolymerization treatments assisted with scCO<sub>2</sub> of mixed plastic waste.
- Task 2.5. Characterization of the degradation products isolated
- Task 8.1 Communication strategy
- Task 8.2 Dissemination activities
- Task 8.3 Exploitation, innovation and IPR management
- Task 8.4: Innovation management

h) Which are the deliverables that your organization will deliver during the project?

- D2.4 Report on depolymerisation and thermodegradation assisted with scCO<sub>2</sub>
- D8.1 Communication Plan (CP)
- D8.2. Preliminary Exploitation plan progress (PEDR).
- D8.3. Mid-Term Exploitation plan progress (PEDR).
- D8.4. Final version of the Plan for the Exploitation and Dissemination of Results (PEDR).

i) Which are the technical objectives that your organization needs to achieve during the project?

Development and optimization of the pre-treatment technologies to enhance the microbial degradation using various microorganisms for individual/mixed non-biodegradable and bio-degradable plastics:

-To design the novel pre-treatment technologies to improve the accessibility of plastic waste for microbial degradation: Pyrolysis, reactive extrusion, depolymerization and thermodegradation assisted with scCO<sub>2</sub>

-To test the effect of various pre-treatment methods to generate carbon source for producing the high value biodegradable products.

-To ensure the commercial scalability and environmental sustainability of pre-treatment methods/technologies

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j) Please provide info regarding the market of the technology that your organization will develop (*size of market, main competitors, costs of services/products etc.* )

AIMPLAS is at the beginning of the value chain and it will have the same market addressed after the microorganism degradation. Some general figures:

Around 25.8 million tonnes of plastic waste are generated in Europe every year<sup>27</sup>. However, less than 30% of such waste is collected for recycling. Environmental legislation is becoming more and more restrictive, and the recently published European strategy for plastics in a circular economy<sup>28</sup> highlights the need to improve the economics and quality of plastics recycling.

## ACADEMIC PARTNER PROFILE

### 3. Partner Profile Information

#### a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

AIMPLAS, Technological Institute of Plastics located in Valencia, is a private, non-profit Association with more than 500 associated companies created in 1990. AIMPLAS is formed by +120 highly skilled professional, more than 65% with a Masters, Engineering or equivalent degree in Chemistry, polymer engineering, materials engineering or equivalent, including 15 PhD.

AIMPLAS' fields of work are related to technological research and development on thermoplastic and thermosetting plastic materials & products, its transformation processes and their recyclability and sustainability. AIMPLAS generates new knowledge and technologies that can be transferred to companies in order to help them to increase their effectiveness and competitiveness.




Nowadays, AIMPLAS is involved in more than 25 European projects and has participated in 77 projects in FP5, FP6, FP7, LIFE+, CIP-EcoInnovation EU Programmes, among others, coordinating 27 of them. At National and Regional levels AIMPLAS participates in around 100 projects yearly.

AIMPLAS, as RTD is focused to help companies in the plastic sector to develop new products and increase their competitiveness through innovation, has more than 20 pilot plants representing the most relevant polymer/plastics/composites production technologies present in the industry nowadays and has state-of-the art test facilities for chemical, optical, morphological, mechanical and physical characterisation. These pilot lines and laboratories are used by many customer's every year allowing them to test new materials, optimize production processes and launch new products to the market, supported by AIMPLAS technical staff, resulting in more than 5000 assays, 170 technical assessments and 120 skills training actions to more than 1500 clients per year. AIMPLAS has state-of-the-art 8500 m2 facilities, including thermoplastics and thermoset pilot plants, analysis and testing laboratories (physical-mechanical, chemical, packaging, automotive and construction) and training areas

b) Relevant elements of the Curriculum Vitae/Biography (*including profile pictures*) of the team responsible for the project implementation. (*Please provide maximum 2 paragraphs per person and refer to the gender of each employee. Thank you*)

<sup>27</sup> <http://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-28-F1-EN-MAIN-PART-1.PDF>

<sup>28</sup> Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. A European Strategy for Plastics in a Circular Economy. COM(2018) 28, 2018

	<p><b>Dr. Belén Monje</b> (female), PhD in Organic Chemistry by the University of Valencia, working in AIMPLAS as researcher at the Chemical Laboratory since 2003. She has more than 13 years of experience in the field of characterization and identification of polymer materials. Moreover, she is a reference in REACH Regulation for plastic users. During this time, she has been involved in many different national and international research projects. In some of them she has been the main technical responsible, as for example in DAFIA-H2020 (Biomacromolecules from municipal solid bio-waste fractions and fish waste for high added value applications) She has 6 scientific publications in organic chemistry, and she participates in different Committees of Standardization.</p>
	<p><b>Dr. Laura Martí</b> (female), PhD in Sustainable Chemistry by the Polytechnic University of Valencia working in AIMPLAS as a researcher in the Synthesis Department. She worked for the Spanish Research Council (CSIC) for 6 years in the field of Fine Chemistry (2009-2015), then she moved to Queen's University of Belfast (2016-2017) to work as Research Associate to carry out investigations for the valorization of the waste cooking oil. She has been involved in National and International projects (CASE project) in the field of valorization of biomass. She has 4 peer-reviewed articles in Scientific Journals and has attended to several Conferences and workshops proceeding in the field of heterogeneous catalysis.</p>
	<p><b>Mrs. Eva Verdejo</b> (female), holds a bachelor's degree in chemistry from the University of Valencia. She joined the Institute in 1993, being the current Head of Department of Sustainability and Industrial Valorization of AIMPLAS. Her expertise is focused on sustainable development, materials from natural resources, biodegradable plastics and recycling. She holds large experience in proposal writing and participation/coordination in relevant national and international R+D projects related to her expertise: circular economy, recycling, biodegradation materials, etc.</p>

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

1. VIDAL R, MOLINER E, MARTIN PP, FITA S, WONNEBERGER M, VERDEJO E, VANFLETEREN F, LAPENA N, GONZALEZ A: Life Cycle Assessment of Novel Aircraft Interior Panels Made From Renewable or Recyclable Polymers With Natural Fiber Reinforcements and Non-Halogenated Flame Retardants. Journal of Industrial Ecology 2018, 22:132-144.
2. VIDAL R, MARTINEZ P, GARRAIN D: Life Cycle Assessment of Composite Materials Made of Recycled Thermoplastics Combined With Rice Husks and Cotton Linters. International Journal of Life Cycle Assessment 2009, 14:73-82.
3. KACHRIMANIDOU V, KOPSAHELIS N, VLYSIDIS A, PAPANIKOLAOU S, KOOKOS IK, MARTINEZ BM, RONDAN MCE, KOUTINAS AA: Downstream Separation of Poly(Hydroxyalkanoates) Using Crude Enzyme Consortia Produced Via Solid State Fermentation Integrated in a Biorefinery Concept. Food and Bioproducts Processing 2016, 100:323-334. Notes: Part Number: A
4. Roig, I., Graf, M., Monje, B., Menes, O., and Eschbach, R. Microwave Curing of Long Fiber Reinforced Composites for Resin Transfer Moulding. 592-602. Notes: Full Source Title: 9th International Conference on Composite Science and Technology: 2020 - Scientific and Industrial Challenges. Publication Year: 2013
5. Waste and Waste Management. Chapter Municipal and Industrial Waste: Sources, Management Practices and Future Challenges. Brenda Bryant, Betty Hall (Editors). April 2018. Authors: Giulio Malucelli, Belén Monje. ISBN: 978-1-53613-441-4

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

-Conferences and trade fairs/exhibitions: EFIB, Compounding World Expo, Conama (Es), Chemplast (Es), JEC (Ge), K fair, FAKUMA and Interpack (Ge), Hispack (Es), European Bioplastics

-Press releases, newspaper articles and other dissemination activities: Plastics News Europe, Interempresas, Bioplastics Magazine, Retema, Renovaveis Magazine, Ambiente Plástico

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

- MW assisted curing.
- Equipment for mechanical, thermal, electrical, rheological and physical tests.
- Dispermat Disperser equipment and laboratory reactors.
- Equipment for large scale synthesis (glass reactor, stainless steel reactor, rotary evapory)
- Equipment for mechanical, thermal, electrical, rheological and physical tests.
- Recycling pilot plant: Grinding facilities, washing equipment with sorting capacity, horizontal centrifuge equipment.
- Pilot plant: Sc-CO<sub>2</sub> cleaning & VOC removal, equipment for extrusion and injection moulding technologies. Pilot-plant twin screw extruder
- FTIR, DSC, TGA, SEM-EDX, GC, HPLC, GPC.
- Minilab extruder Haake.

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## INDUSTRY PARTNER PROFILE

### Section 1. General Partner Information

1. Partner name: **Avecom**
2. Partner Website: <https://avecom.be/>
3. Participant Identification Code (PIC) No: 999693835
4. Contact person name and email address: [thijs.demulder@avecom.be](mailto:thijs.demulder@avecom.be)
5. Position in organization: **R&D Engineer**
6. Department name: **R&D**
7. Average Person Month Rate in the organization: **€5,350**



## INDUSTRY PARTNER PROFILE



a) Which is the role of your organization in the proposal? (*Explain in 2 sentences. Thank you.*)

Avecom will construct a pilot scale reactor setup based on the specification on lab-scale trials and using the strains selected in earlier WP's.

b) Which is the current state of the art of the technology you will introduce/progress during the project? *Please provide an overview of commercial solutions and initiatives in scientific community in the domain, patents and projects according to your knowledge*

After being initially developed in the 1970's, the production of microbial protein (MP) was 're-invented' recently, due to higher protein prices, rising awareness of the importance of sustainability and a harder push of governments towards alternative protein sources. MP is already on the market and used in different feed and food applications, e.g. FeedKind by Calista (CA, USA) or UniProtein by Unibio (UK). Yet, these products only use specific strains of bacteria as end product, rendering cultivation conditions challenging, and virtually excluding their use for heterogeneous bio-waste valorisation, such as vegetable residues. The proof-of-concept of MP production from food industry wastewater was demonstrated by the Avecom, using an optimised culture of bacteria, a 'microbiome'. By starting from a rich inoculum and steering the community to specific functionalities by imposing selection pressure and creating an optimal ambient environment, the best performing bacteria are enriched in an optimally functioning microbiome. This selection approach is currently being used by Avecom to produce microbial protein rich in Polyhydroxyalkanoates (PHA) in collaboration with partner NOVA. Various tests were conducted at lab and demonstrator scale to steer the microbiome by changing relevant parameters such as chemical oxygen demand (COD), loading rate and residence time, and to determine the optimal parameters to ensure a high-quality and consistent process. The use of a microbiome opens possibilities for the production of MP on more complex and varying streams, but demonstration of MP production from vegetable residue mixtures (or carboxylic acids in general) did not take place yet. Pilot demonstrations on food industry wastewater have furthermore resulted in the identification of several bottlenecks. For instance, MP is hard to dewater because it requires expensive and extensive dewatering. Moreover, the microbiome might have changing characteristics due to fluctuation of the wastewater composition feeding the system.

Avecom is also involved in projects focussed on optimizing microbial processes for the degradation of chlorinated compounds (soil and water remediation) and the production of PHA by improving reactor design and steering the microbial community to specific functionalities.

However, using (by)products of depolymerization of pre-treated plastics for the production of microbial biomass rich in PHA has not been explored by Avecom.

Avecom will introduce its knowledge and expertise in the microbiome engineering (WP5) and the pilot design and operation (WP6) in the BioICEP project.

c) Does your team have recent relevant scientific papers in this domain? (no more than 4 years old) Please provide a full list

1. Pikaar I., Matassa S., Bodirsky B., Weindl I., Humpenöder F., Rabaey K., Boon N., Bruschi M., Yuan Z., van Zanten H., Herrero M., Verstraete W. and Popp A. (2018) Decoupling Livestock from Land Use through Industrial Feed Production Pathways *Environmental Science & Technology*. 2018 52 (13): 7351-7359
2. Matassa S., Verstraete W., Pikaar I. and Boon N. (2016). Autotrophic nitrogen assimilation and carbon capture for microbial protein production by a novel enrichment of hydrogen-oxidizing bacteria. *Water Res.* 101: 137-146,
3. Matassa S., Boon N., Pikaar I. and Verstraete W. (2016). Microbial protein: future sustainable food supply route with low environmental footprint. *Microbial Biotech.* 9(5), 568-575
4. Patent application on reactor technology for autotrophic microbial protein using hydrogen: patent application n° P112565EP00 'Bioreactor for aerobic hydrogenotrophic fermentation'.
5. Patent application on heterotrophic single cell protein production from potato processing water: patent application n° BE2014/0641 "Enhanced method for recovery of proteins from process water.

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

1. Power-to-protein (Partner) Technical and economic feasibility of the Power-to-Protein concept in the water cycle of the city of Amsterdam. The basis is a highly efficient microbial re-synthesis process with a mixed culture of bacteria that use hydrogen as an energy source. – TKI Water technology Programme (2015-2018). Web-site: <https://www.powertoprotein.eu/>

2. *MicroNOD (Partner) Microbial Nutrients On Demand: microbial immobilization and release of nutrients as an innovative and sustainable upgrade of waste products into tailor-made slow release organic fertilizers - MIP-ICON project, Flanders Cleantech research for transition (2015-2017). Web-site: <https://www.micronod.be/>*
3. *LVM-Biocells (Partner) Using hydrogeobiocells (HGBcells) for the in situ biological treatment of CAH contaminated groundwater in areas with low hydraulic gradients - EU-LIFE08 ENV/B/000046" – Life + program 2008 (2010-2016). Web-site: <https://www.lvm-biocells.be/>*
4. *YPACK (Partner) High Performance Polyhydroxyalkanoates based Packaging to minimise Food Waste – H2020-SFS-2017-1 (2018-2021). Web-site: <https://www.ypack.eu/>*
5. *Biotreat(Partner) Biotreatment of drinking water resources polluted by pesticides, pharmaceuticals and other micropollutants - FP7 (2011-2014). Web-site: <http://www.biotreat-eu.org/>*

e) Does your company provide related services or products as the ones mentioned in the project? *Please provide more information regarding the content and the clientele receiving this kind of services/products. Thank you.*

Avecom provides R&D support and services for companies and industrial partners such as microbial process optimization and reactor scale-up. In particular, respiration tests are carried out to determine rate of degradation of plastics on soils samples.

f) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

Avecom will receive the results of the lab-scale experiments and strain characterisation, performed by the various project partners, and used this information to create a design of a demonstration scale reactor setup. The main scientific and technical challenge is to upscale and combine these different scientific outcomes into a single solution/process, which should be economically feasible (in terms of CAPEX and OPEX), safe and functional.

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- Implementing a lab-scale reactor fed with pretreated plastics (added with N/P sources) and inoculated with a rich but undefined inoculum collected from relevant environments. The reactor will be operated to create a microbial community optimized for the utilisation of the carbon in pretreated plastics.
- Design and construction of a demonstration set-up in a industrial environment.

h) Which are the deliverables that your organization will deliver during the project?

- Bacteria microbiome capable of degrading plastics/products of depolymerization.
- Pilot design based on the specifications provided by the consortium partners.
- Pilot construction and operation for demonstration of BioICEP concept.

i) Which are the technical objectives that your organization needs to achieve during the project?

- Development of a microbiome capable of degrade PE/PU depolymerization products provided by Partner X.
- Demonstration of the BioICEP concept at TRL4-5. A demonstration plant will be design, build and operated following specifications provided by other partners.

j) *Please provide info regarding the market of the technology that your organization will develop. Thank you. (size of market, main competitors, costs of services/products etc.)*

Business approach: Avecom intends to industrialize specialized microbiomes for degradation of depolymerized plastics and for start up of production of microbial rich in PHA. The main commercial objective is to develop the technology and license the process. In addition, Avecom plans to sell optimized microbiomes needed to (i) to start up the degradation of products of depolymerization and (ii) to start up the production of biomass rich in PHA. This microbiomes will ensures that the characteristics and functionality of the microbial biomass remains standard through the process. This minimises the investment risk for Avecom. Besides the licensing agreement for the product, we will also sell the microbiome to start up the full scale of reactors.

1. Microbiome for treating depolymerized products
2. Biomass rich in PHA for bioplastics production

**Existing alternatives and competitors:** Naturflex™ is a product of Innovia Films (UK) which is a biobased, biodegradable and compostable PLA film. However, barrier properties are obtained by the use of polyvinylidene chloride (PVdC). Nativia™ packaging is a product of Taghleef Industries (Dubai U.A.E.) is a biobased, biodegradable and compostable PLA film. Sustain™ packaging is a product of London Bio Packaging made from plants and compostable. The materials used are PLA, Starch, sugar bagasse and the type of packaging is used for containers, cups, and boxes. 100% Biodegradable Food Packaging from Green Home is bio-packaging. Potential PHA based plastics should have thermal and barrier properties: three times higher thermal stability (150°C) than current biobased plastics (polylactic acid –PLA- show just 50°C); and barrier properties against oxygen two times better than petrochemical polyester (PET) and forty times better than polyethylene (PE). Moreover, PHB is biodegradable in soil, unlike PLA, which only industrially compostable. PLA and starch-based plastics currently available in the market mostly come from corn or other crops, making therefore food price higher.

**Market size:** With a global production of 4.2 million tonnes in 2016, bioplastic market is expected to grow by 20% every year, to reach a production capacity of 6 million tonnes in 2021, being flexible packaging the application that is growing faster (it accounts for 12.28% of the bioplastic's market (See fig. below: Foreseen global bioplastics' production capacity. Source: European Bioplastic Association (EBA)).

If another end-product is pushed forward during the project, such as rhamnolipid, nanocellulose, succinate, a study should be performed to investigate the potential market size and to draw a business approach.

## INDUSTRY PARTNER PROFILE

### 3. Partner Profile Information

#### a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

Avecom (BE) is an innovative SME specialised in steering and optimizing microbial processes in environmental and industrial applications. The company originated 20 years ago as a spin-off of the Faculty of Bioscience Engineering (University of Ghent). Research has therefore always been the main core business of Avecom. This is well demonstrated by the active participation of Avecom in various national and international research projects, where it represents one of the leading innovators in the field of microbial and environmental biotechnology. Avecom deliberately focuses on translating and combining high level innovation in practical and hands-on projects. Selecting strategic partners from an extensive and well-established network, Avecom provides a holistic approach to lead ambitious projects to success, helping to build the future of a more environmentally and economically sustainable society. The research activities of Avecom include both the use of conventional techniques in a new framework and the development of new technologies for existing and persistent problems. The projects at Avecom span from small feasibility studies to large-scale research and development projects, performed for and with partners from industry, governmental agencies and academia. Since it was founded, Avecom has specialized in conducting research on aerobic and anaerobic wastewater treatment. Additionally, Avecom also has strong expertise in soil treatment and it has become a specialist in the microbiological decontamination of volatile organochlorine compounds (VOC), with the soil screened using microcosm tests. Avecom recently patented a new biotechnological process for the production of microbial protein from food processing water and is active in the research and development concerning side stream valorisation by means of microbial protein production.

b) Relevant elements of the Curriculum Vitae/Biography *(including profile pictures)* of the team responsible for the project implementation *(Please provide maximum two paragraphs per person and refer to the gender of each employee. Thank you.)*

Dr. Thijs De Mulder (male) has graduated as bioscience engineer in 2013 and started a PhD at the Flemish institute for agriculture, fisheries and food research (ILVO) studying the microbial communities in pigs and cattle. After his PhD, he



Dr. Carlos Zamalloa (male) earned his PhD at CMET in 2012. He worked as postdoctoral researcher at the University of Minnesota (USA) for 3.5 years. He also was invited lecturer at the National University of Engineering (Peru). His work has been focused on microbial driven process development with a special interest in resource recovery. He co-supervised 7 Master students and published more than 10 journal articles. Since January 2018, Carlos works as Research and Development Scientist at Avecom focusing on autotrophic and heterotrophic microbial protein and PHA production.

Senior project engineer, Mariane Van Wambeke (female) has been involved in R&D projects at Avecom for more than 30 years. She has become an expert in the domain of wastewater treatment, anaerobic digestion and heterotrophic microbial protein production. In the last 2 years she was involved in the training of 2 PhDs within two different European ITN at Avecom.

Prof. em. Willy Verstraete (male) is CEO and scientific coordinator of Avecom. Previously, he was head of the Laboratory of Microbial Ecology and Technology (LabMET - currently CMET, 1979-2011) and Professor in microbial ecology and technology at Ghent University. His R&D has a central theme: processes mediated by microbial mixed cultures. Willy Verstraete has more than 800 peer reviewed papers with a h-index of 109 (Scopus), he has supervised 35 PhD students in the last 10 years.

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

Publications already provided in 2c

Avecom has a several commercial product lines. The first offers nitrifying organisms for bioremediation in aquaculture, ponds, aquaria. The second offers microbiomes specialised in the degradation of specific chlorinated compounds for bioremediation of contaminated soil. Further, Avecom is at pilot stage in an industrial project to create microbial protein for potato waste streams.

Furthermore, Avecom offers R&D services to industry to create and optimize microbial processes.

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

**Not relevant**

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

Avecom's infrastructure includes pilot/industrial production facilities including several fully automated reactors for the production and processing of different microbial products such as single cell protein (20-2500 L reactors). In addition, Avecom has a fully equipped service and R&D laboratory to fully characterize and quantify intermediate process samples as well as the main chemical and physico-chemical characteristics of final products. Analytical equipment includes Kjeltex digester and analyser (for protein analysis), chemical oxygen demand (COD) digester and analyser, gas chromatography with FID and TCD, etc. Furthermore, Avecom's laboratory is capable to identify bacterial species using the classic methods and high throughput sequence techniques such as amplicon sequencing. Moreover, Avecom has recently included the engineering of microbiomes to achieve very specific activities in our portfolio of services

## ACADEMIC PARTNER PROFILE

### Section 1. General Partner Information

1. Partner name: Clausthal University of Technology (Technische Universität Clausthal)
2. Partner Website: [www.tu-clausthal.de](http://www.tu-clausthal.de)
3. Participant Identification Code (PIC) No: 999865913
4. Contact person name and email address:
  - a) Dr. Georgia Sourkouni [georgia.sourkouni-argirusi@tu-clausthal.de](mailto:georgia.sourkouni-argirusi@tu-clausthal.de)
  - b) Prof. Christos Argirusis [christos.argirusis@tu-clausthal.de](mailto:christos.argirusis@tu-clausthal.de)
5. Position in organization: Senior Researcher
6. Department name: Clausthal Centre of Materials Technology
7. Average Person Month Rate in the organization: 6900 €





## Section 2. Technical Part Contribution

a) Which is the role of your organization in the proposal? *(Please explain in two sentences. Thank you.)*

CUT will participate in WP2 and will work on the pre-treatment of plastics and in WP4 on the elucidation of the mechanism of the enzymatic and/or microbial attack on pristine or pre-treated plastics in close co-operation with NTUA.

b) Which is the current state of the art of the technology you will introduce/progress during the project? Please provide a review of the recent scientific papers in the domain, patents and projects according to your knowledge. Thank you.

To the best of our knowledge, there is information on that issue only from the group in NTUA for poly(ethylen terephthalate) (PET) [Kanelli M, Vasilakos S, Nikolaivits E, Ladas S, Christakopoulos P, Topakas E (2015) Surface modification of poly(ethylene terephthalate) (PET) fibers by a cutinase from *Fusarium oxysporum*. *Process Biochem* 50:1885–1892] on polylactic acid [Lee SH, Song WS (2013) Modification of polylactic acid fabric by two lipolytic enzyme hydrolysis. *Text Res J* 83:229–237] and recent works on the modification of nanocellulose by CUT and NTUA [Anthi Karnaouri et al. LPMO-assisted preparation of oxidized nanocellulose with high carboxyl content from tunicate biomass, *Symposium on Biotechnology for Fuels and Chemicals*, April 28-May 1, 2019, Seattle, USA].

c) Does your team have recent scientific papers in this domain? (no more than 4 years) (Please provide a full list. Thank you)

- LPMO-assisted preparation of oxidized nanocellulose with high carboxyl content from tunicate biomass A. Karnaouri, B. Jalvo Sánchez, Ph. Moritz, L. Matsakas, U. Rova, A. Mathew, O. Höfft, G. Sourkouni, W. Maus-Friedrichs, P. Christakopoulos

Symposium on Biotechnology for Fuels and Chemicals, April 28-May 1, 2019, Seattle, USA

- Copper NPs decorated titania: A novel synthesis by high energy US with a study of the photocatalytic activity under visible light  
M. Stucchi, C.L. Bianchi, C. Pirola, G. Cerrato, S. Morandi, Chr. Argiris, G. Sourkouni, A. Naldoni, V. Capucci  
*Ultrasonics Sonochemistry* 31 (2016) 295-301
- Interaction mechanism of hydrogen storage materials with layer-by-layer applied protective polyelectrolyte coatings

Georgia Sourkouni, Florian Voigts, Jan C. Namyslo, Sebastian Dahle, Wolfgang Maus-Friedrichs, Christos Argiris

*Int.J. Hydr. Energy* (39) 2014 14834-14842

- Sonochemistry in the service of SOFC research  
Petros M. Sakkas, Oliver Schneider, Georgia Sourkouni, Christos Argiris  
*Ultrasonics Sonochemistry* 21 (2014) 1939–1947
- Chemical improvement of surfaces. Part 4: Significantly Enhanced Hydrophobicity of Wood by Covalent Modification with p-Sily-functionalized Benzoates  
C. Kaldun, S. Dahle, W. Maus-Friedrichs and D. E. Kaufmann  
*Holzforschung*, DOI: 10.1515/hf-2015-0036, 2015
- Characterisation of PMMA/ATH layers realised by means of atmospheric pressure plasma powder deposition  
L. Wallenhorst, S. Dahle, M. Vovk, L. Wurlitzer, L. Loewenthal, N. Mainusch, C. Gerhard and W. Viöl  
*Advances in Condensed Matter Physics*, DOI: 10.1155/2015/980482, 2015
- Plasma chemical and chemical functionalization of polystyrene colloidal systems  
L. Wegewitz, A. Prowald, J. Meuthen, S. Dahle, O. Höfft, F. Endres and W. Maus-Friedrichs  
*Physical Chemistry Chemical Physics*, 16, 18261-18267, DOI: 10.1039/C4CP01932F, 2014
- Adsorption analysis of thin films of terephthalic acid on Au and Al studied by MIES, UPS and XPS  
M. Marschewski, C. Otto, L. Wegewitz, O. Höfft, A. Schmidt and W. Maus-Friedrichs  
*Applied Surface Science*, 2014

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

- FP7 Project “ROBANODE”: We have used high power ultrasounds to sonochemically and sonoelectrochemically convert precursors to produce metal-ceramic nano-composites as catalysts for fuel cells. Associated with document Ref. Ares(2019)6080743 - 01/10/2019
- Several national projects on the sonochemically assisted materials preparation.
- Several National projects on the surface modification and characterization of ceramics, plastics, wood etc.

e) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

The first goal of CUT is to investigate the pre-treatment of plastics using high-power ultrasound, where we will vary parameters such as time, temperature, solvent, and power intensity followed by complete characterization of the material to prove its modification through the pre-treatment.

The second and more important goal of CUT is to elucidate the mechanism of the enzymatic and/or bacterial attack on the plastics with and without pre-treatment. To the best of our knowledge, there is information on that issue only from the group in NTUA for poly(ethylen terephthalate) (PET) [Kanelli M, Vasilakos S, Nikolaivits E, Ladas S, Christakopoulos P, Topakas E (2015) Surface modification of poly(ethylene terephthalate) (PET) fibers by a cutinase from *Fusarium oxysporum*. *Process Biochem* 50:1885–1892], on polylactic acid [Lee SH, Song WS (2013) Modification of polylactic acid fabric by two lipolytic enzyme hydrolysis. *Text Res J* 83:229–237] and on the modification of nanocellulose by CUT and NTUA [Anthi Karnaouri et al. LPMO-assisted preparation of oxidized nanocellulose with high carboxyl content from tunicate biomass]. We will investigate this issue in cooperation with the National Technical University of Athens (Prof. Topakas) using the advanced surface characterization techniques at the CUT on samples modified by the enzymes using methods developed by NTUA.

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- WP2

#### Description of Work :

Plastics pre-treatment using ultrasounds (US) and characterization regarding their crystallographic phase and its stability. Power ultrasounds will be used in order to pre-treat the plastics, which then will be more prone to degrade in an microbial and/or enzymatic step.

The following parameters will be varied: ultrasonic intensity, frequency, initial concentration of polymer solution, type of solvent, pH, and operating temperature. Further, a crucial parameter will be the type of the plastic to be treated as its molecular weight and chain constituents play a crucial role. Therefore, all procedures described in the tasks below will be applied to all plastic sortments and mixtures in the BioICEPT proposal.

#### Tasks

##### **Task 2.1 (CUT): Variation of the ultrasound frequency and respective intensity**

**Sub-Task 2.1.1 (CUT): Variation of the ultrasound frequency and respective intensity:** The frequency of the used ultrasound will be varied between low frequency (20 – 40 kHz) and high frequency (600 – 1200 kHz). The effect of the US frequency will be characterized as described in Task 2.3.

**Sub-Task 2.1.1 (CUT): The interaction of the plastics with US in presence of additional dissolved oxygen:** In a similar step as in Sub-Task 2.1.1 the interaction of oxygen with the plastics under sonochemical conditions will be performed and the possible influence of oxygen radicals on the plastic degradation during pre-treatment will be investigated.

**Task 2.2 (CUT): Variation of solvent, pH and additives:** As sonochemistry is considered a green chemistry process the main goal of BioICEPT is to use water as solvent for the pre-treatment. Salt could be tested as additive to check the case of pre-treatment of plastics in sea water.

##### **Task 2.3 (CUT): Characterization of the pre-treated plastics (in Tasks 2.1 and 2.2)**

The pre-treated plastics will be characterized using HRTEM with EDX, XPS/UPS and MIES/UPS. For the investigation of surface structure may be also applied methods of AFM and confocal laser microscopy. The molecular weight of the treated samples will be estimated as well (NTUA, TUC).

Fragments from the plastics released during pretreatment will be characterized using GPC and/or HPLC.

**Task 4.1 (CUT, NTUA):** Surface properties of the pre-treated plastics (in Tasks 2.1 and 2.2) and relation to enzymatic attack mechanism:

Surface properties of the pre-treated plastics will be measured using very sensitive analytical methods as Ultraviolet Photoelectron Spectroscopy in combination with metastable (Helium atoms) Induced Electronspectroscopy (UPS/MIES) in order to characterize the outermost surface groups and so to elucidate the mechanism of the enzymatic attack on the surface. This Task will be performed in cooperation with the group in NTUA, who will provide (pre-treated samples from CUT) after enzymatic attack for certain time(s).

h) Which are the deliverables that your organization will deliver during the project?

**D2.1:** Report on the influence of the US frequency (M9)

**D2.2:** Report on the interaction of the US in presence of oxygen (M12)

**D2.3:** Report on the chemical composition of fragments produced during pre-treatment (M9-M24)

**D2.4:** Report on the influence of temperature, pH and additives (M9-30)

**D4.1:** Report on the mechanism of enzymatic and/or microbial attack on the pre-treated plastics (M18-M36)

i) Which are the technical objectives that your organization needs to achieve during the project?

The goal is to obtain partly degraded plastics, which in a second step can be further degraded via microbial and/or enzymatic processes.

The second objective is to elucidate the enzymatic attack mechanism on each plastic sort. This will be useful in order to be able to define specific enzymes for each type of plastic to be degraded.

j) Please provide info regarding the market of the technology that your organization will develop (*size of market, main competitors, costs of services/products etc.*)

CUT will perform mainly basic research and thus will not develop market ready technologies. The target is the same as for the overall project, namely to develop a complete procedure for the degradation of plastics and the re-use of the degradation products.

## ACADEMIC PARTNER PROFILE

### 3. Partner Profile Information

a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

Clausthal University of Technology (CUT) is an internationally renowned institution with strong regional ties. The University has strong traditions of quality education recognized and valued by many national as well as international companies. Research and education at Clausthal University of Technology are currently focused on Energy and Raw Materials, Natural Science and Materials Science, Economics, Mathematics, Computer Science, Mechanical Engineering and Process Engineering. In three innovative centers, the Energy Research Center Niedersachsen (EFZN), the Clausthal Centre of Material Technology (CZM) and the Center of Simulation (SWZ), we aim to link applied research in natural science, engineering and economics.

TUCs group of Functional Layers integrated in the Faculty of Natural and Materials Sciences and the Clausthal Research Centre for Materials Technology, has a long and successful experience in co-ordination and participation in European Research projects. TUC has high skills in production, characterisation and application of polymers, metal alloys, ceramic materials, and in modern preparation methods for materials. Sonochemical and sono-electrochemical methods are used for the preparation of nanosized materials for application e.g. as electrocatalysts. Further, we have long expertise in the

b) Relevant elements of the Curriculum Vitae/Biography (including profile pictures) of the team responsible for the project implementation. (Please provide maximum 2 paragraphs per person and refer to the gender of each employee. Thank you)

Dr. Georgia Sourkouni (female), is leader of the functional layers group at the Clausthal Center of Materials Technology. He is a chemist with more than 20 years of experience in acquisition and coordination of scientific projects (both national and European) with focus in materials science and organic chemistry. Dr. Sourkouni will be the responsible person for the BioICEP project and will be coordinating the workflow at CUT.

Prof. Christos Argiris (male): He is a chemist with more than 25 years of experience in acquisition and coordination of scientific projects. He has expertise in materials science with emphasis on sono-electrochemical methods. He is currently visiting Professor and member of the Clausthal Centre of Materials Technology. Prof. Argiris will be responsible for the technical implementation of the ultrasound assisted pre-treatment of the plastics.

Prof. Wolfgang Maus-Friedrichs (male): Prof. at the TUC is leading the group of Surface Science in the Institute of Energy Research and Physical Technology and member of the Clausthal Center of Materials Technology. He has 25 years of experience in Materials Physics with focus on surface related problems. He is specialist in analytical techniques like Photo-Electrons-Spectroscopy and functionalization of surfaces using atmospheric plasma techniques. Prof. Maus-Friedrichs will be responsible for the technical implementation of the surface characterization of the samples.

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

1. Plasma chemical and chemical functionalization of polystyrene colloidal systems  
L. Wegewitz, A. Prowald, J. Meuthen, S. Dahle, O. Höfft, F. Endres and W. Maus-Friedrichs  
Physical Chemistry Chemical Physics, 16, 18261-18267, DOI: 10.1039/C4CP01932F, 2014
2. Adsorption analysis of thin films of terephthalic acid on Au and Al studied by MIES, UPS and XPS  
M. Marschewski, C. Otto, L. Wegewitz, O. Höfft, A. Schmidt and W. Maus-Friedrichs  
Applied Surface Science, 2014
3. Interaction mechanism of hydrogen storage materials with layer-by-layer applied protective polyelectrolyte coatings

Georgia Sourkouni, Florian Voigts, Jan C. Namyslo, Sebastian Dahle, Wolfgang Maus-Friedrichs, Christos Argiris

Int.J. Hydr. Energy (39) 2014 14834-14842

4. Sonochemistry in the service of SOFC research  
Petros M. Sakkas, Oliver Schneider, Georgia Sourkouni, Christos Argiris  
Ultrasonics Sonochemistry 21 (2014) 1939–1947
5. LPMO-assisted preparation of oxidized nanocellulose with high carboxyl content from tunicate biomass A.  
Karnaouri, B. Jalvo Sánchez, Ph. Moritz, L. Matsakas, U. Rova, A. Mathew, O. Höfft, G. Sourkouni, W. Maus-Friedrichs, P. Christakopoulos

Accepted for presentation on the Symposium on Biotechnology for Fuels and Chemicals, April 28-May 1, 2019, Seattle, USA

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

1. European Society of Sonochemistry Meeting, 2020
2. Symposium on Biotechnology for Fuels and Chemicals 2021.

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

- Several high power ultrasound devices with low or high frequency for the (pre)-treatment of the plastics
- Two systems performing photoelectron spectroscopy (XPS / UPS / MIES)
- Micro computed tomography
- High resolution FE-SEM with EDS
- All other characterization methods for surfaces and thin films like AES, LEED, QMS, AFM, STM



## Section 1. General Partner Information

1. Partner name: **INSTITUT ZA MOLEKULARNU GENETIKU I GENETICKO INZENJERSTVO**  
**Institute of Molecular Genetics and Genetic Engineering (IMGGE)**
2. Partner Website: <https://imgge.bg.ac.rs/en/>
3. Participant Identification Code (PIC) No: **986427921**
4. Contact person name and email address: Jasmina Nikodinovic-Runic
5. Position in organization: Principal Research Fellow
6. Department name: Laboratory for Microbial Molecular Genetics and Ecology
7. Average Person Month Rate in the organization: 1000 eur



## ACADEMIC PARTNER PROFILE

## Section 2. Technical Part Contribution

a) Which is the role of your organization in the proposal? (Please explain in two sentences. Thank you.)

IMGGE will lead WP4 (Enzymatic and Biocatalytic Solutions) and will also contribute to WP1 (Co-ordination and management), WP3 and WP5 (Development of Strains and Microbial Consortia for plastic degradation), WP6 (Valorisation) and WP8 (Dissemination and exploitation).

The group of Dr Nikodinovic-Runic will apply its expertise on the preparation of synthetic model compounds (to be supplied to WP3 and WP5) to allow more efficient screening of microbial strains and enzymes for hydrolysis of various plastic polymers, in development of enzymatic processes for the breakdown/depolymerisation of mixed plastic waste materials (WP4), and on utilisation of plastic-derived monomeric carbon sources for value added products (WP6). Protein engineering and microbial strain development is another key strength of the Dr Nikodinovic-Runic group, which will be employed to enhance productivity of microbial depolymerisation of plastic polymers. IMGGE will also take an active role in dissemination activities (WP8).


b) Which is the current state of the art of the technology you will introduce/progress during the project? Please provide a review of the recent scientific papers in the domain, patents and projects according to your knowledge. Thank you.

Scientific literature on enzymes and microbes degrading various single plastic polymers are abundant in the literature. However, these still need to be judged by whether it can degrade real-world plastic waste and do so efficiently.

The most important refs:

- Wei, R.; Zimmermann, W., *Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we?* *Microb Biotechnol* **2017**, *10* (6), 1308-1322.
- Wierckx, N.; Prieto, M. A.; Pomposiello, P.; de Lorenzo, V.; O'Connor, K.; Blank, L. M., *Plastic waste as a novel substrate for industrial biotechnology* *Microbial Biotechnology* **2015**, *8* (6), 900-903.
- Wei, R.; Zimmermann, W., *Biocatalysis as a green route for recycling the recalcitrant plastic polyethylene terephthalate* *Microbial Biotechnol* **2017**, *10* (6), 1302-1307.
- Hajighasemi, M.; Nocek, B. P.; Tchigvintsev, A.; Brown, G.; Flick, R.; Xu, X.; Cui, H.; Hai, T.; Joachimiak, A.; Golyshin, P. N.; Savchenko, A.; Edwards, E. A.; Yakunin, A. F., *Biochemical and structural insights into enzymatic depolymerization of polylactic acid and other polyesters by microbial carboxylesterases* *Biomacromolecules* **2016**, *17* (6), 2027-39.
- Han, X.; Liu, W.; Huang, J. W.; Ma, J.; Zheng, Y.; Ko, T. P.; Xu, L.; Cheng, Y. S.; Chen, C. C.; Guo, R. T., *Structural insight into catalytic mechanism of PET hydrolase* *Nature Communications* **2017**, *8* (1), 2106.



- Pellis, A.; Cantone, S.; Ebert, C.; Gardossi, L., *Evolving biocatalysis to meet bioeconomy challenges and opportunities*. *New Biotechnology* **2018**, *40* (Pt A), 154-169.  Associated with document Ref: Ares(2019)6080743 - 01/10/2019

Any enzyme that can break up plastic will be In 2016, researchers in Japan tested sludge from a recycling plant and uncovered a microbe that could completely break down films of PET to CO and H<sub>2</sub>O, a feat that was a step above partial degradations reported previously. From that microbe, the scientists plucked two enzymes that degraded PET to its monomers of terephthalic acid and ethylene glycol (*Science* 2016, DOI: [10.1126/science.aad6359](https://doi.org/10.1126/science.aad6359)).

Enzymes capable of depolymerisation of single target plastic materials have been identified. Most reported cases of enzymes and microorganisms degrading plastic are incomplete and slow. Making these processes faster and more efficient is not trivial.

We will screen wide panel of known enzymes and the ones developed by partners in the consortium (100+) for the ability to hydrolyze standard (i.e. emulsified polymeric materials) as well as model molecules resembling dimers and oligomers of the selected plastic materials, improving our chances to detect enhanced or novel activities suitable for the plastic depolymerisations.

Selection of enzymes will be made according to performance parameters.

Subset of these enzymes will be subjected to directed evolution experiments for enhancement of the activity.

We will also concentrate on using mixture of substrates and mixture of enzymes to obtain synergistic effects.

We will use standard engineering techniques to generate improved biocatalysts.

c) Does your team have recent scientific papers in this domain? (no more than 4 years) (Please provide a full list. Thank you)

- Spasic J, Mandic M, Djokic L, Nikodinovic-Runic J. (2018) Streptomyces spp. in the biocatalysis toolbox. *Appl Microbiol Biotechnol* 102(8):3513-3536.
- Spasic J, Mandic M, Radivojevic J, Jeremic S, Vasiljevic B, Nikodinovic-Runic J, Djokic L. (2018) Biocatalytic potential of Streptomyces spp. isolates from rhizosphere of plants and mycorrhizosphere of fungi. *Biotechnol Appl Biochem*. 65(6):822-833
- Jeremic S, Beškoski VP, Djokic L, Vasiljevic B, Vrvic MM, Avdalovic J, Gojgic Cvijovic G, Beškoski LS, Nikodinovic-Runic J. (2016) Interactions of the metal tolerant heterotrophic microorganisms and iron oxidizing autotrophic bacteria from sulphidic mine environment during bioleaching experiments. *J Environ Manage*. 2016 172:151-61.
- Djokic L, Spasic J, Jeremic S, Vasiljevic B, Prodanovic O, Prodanovic R, Nikodinovic-Runic J. Immobilization of Escherichia coli cells expressing 4-oxalocrotonate tautomerase for improved biotransformation of  $\beta$ -nitrostyrene. (2015) *Bioprocess Biosyst Eng*.38(12):2389-95.
- Radivojevic J, Skaro S, Senerovic L, Vasiljevic B, Guzik M, Kenny ST, Maslak V, Nikodinovic-Runic J, O'Connor KE. Polyhydroxyalkanoate-based 3-hydroxyoctanoic acid and its derivatives as a platform of bioactive compounds.(2016) *Appl Microbiol Biotechnol*.;100(1):161-72.
- Narancic T, Davis R, Nikodinovic-Runic J, O' Connor KE. (2015) Recent developments in biocatalysis beyond the laboratory. *Biotechnol Lett*. 37(5):943-54.
- Jovanovic P, Jeremic S, Djokic L, Savic V, Radivojevic J, Maslak V, Ivkovic B, Vasiljevic B, Nikodinovic-Runic J. (2014) Chemoselective biocatalytic reduction of conjugated nitroalkenes: new application for an Escherichia coli BL21(DE3) expression strain. *Enzyme Microb Technol*. 60:16-23.
- Guzik MW, Kenny ST, Duane GF, Casey E, Woods T, Babu RP, Nikodinovic-Runic J, Murray M, O'Connor KE. (2014) Conversion of post consumer polyethylene to the biodegradable polymer polyhydroxyalkanoate. *Appl Microbiol Biotechnol* 98(9):4223-32.
- Nikodinovic-Runic J, Guzik M, Kenny ST, Babu R, Werker A, O Connor KE. (2013) Carbon-rich wastes as feedstocks for biodegradable polymer (polyhydroxyalkanoate) production using bacteria. *Adv Appl Microbiol*. 84:139-200.
- Narancic T, Radivojevic J, Jovanovic P, Francuski D, Bigovic M, Maslak V, Savic V, Vasiljevic B, O'Connor KE, Nikodinovic-Runic J. (2013) Highly efficient Michael-type addition of acetaldehyde to  $\beta$ -nitrostyrenes by whole resting cells of Escherichia coli expressing 4-oxalocrotonate tautomerase. *Bioresour Technol*.142:462-8.

- Molloy S, Nikodinovic-Runic J, Martin LB, Hartmann H, Solano F, Decker H, O'Connor KE. (2013) Engineering of a bacterial tyrosinase for improved catalytic efficiency towards L-tyrosine using random and site directed mutagenesis approaches. Biotechnol Bioeng. 110(7):1849-57.

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

- 2018 – Research project: ‘The upcycling of waste plastic packaging material to a biodegradable plastic: Waste to value added product’- Green Innovation Vouchers Scheme for Serbia, European Bank for Reconstruction and Development (EBRD), Austrian DRIVE (Delivering Resource Efficiency InVEstments) Programme (proof of concept study)
- 2011-2019 National project: ‘Microbial diversity study and characterization of beneficial environmental microorganisms’ Ministry of Education, Science and Technological Development (Grant No 173048)

e) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

Development of the efficient enzymatic and biocatalytic solution(s) for depolymerisation of variety of plastic polymers and mixes of thereof. The specific challenge will pose the complex nature of the substrates.

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- Biodiscovery screen of existing and new microbial biobanks.
- Isolation of new microbes and biobank enrichment
- Construction of a single microbial platform for boosting plastics degradation capacity
- Liquid media cultivation with standard plastics
- Liquid media cultivation with pre-treated plastics
- quantitative/qualitative analysis of plastic breakdown potential and dynamics
- Identification of potential PHB, rhamnolipid and nanocellulose producers

h) Which are the deliverables that your organization will deliver during the project?

- Isolation and establishment of new biobank microbial strains
- Identification of microbial degraders of plastics from existing and new biobanks
- Generation of novel strains with boosted plastic degradation capacities
- Establishment of liquid cultivation conditions for pre-identified degraders on standard and pre-treated plastics
- Characterization of depolymerase enzyme activities after liquid cultivation
- Quantitative and qualitative characterisation of plastic breakdown potential and dynamics
- Identification of PHB, rhamnolipids and nanocellulose producers
- Consolidated identification and recommendation of the best microbes and their growth conditions to support WP4, WP5, and WP6 for various up-scaling optimizations.
- Sharing of identified strains, their growth conditions for consortia optimization after signing of appropriate material transfer agreement.

i) Which are the technical objectives that your organization needs to achieve during the project?

- Produce pure enzymes and screen them for the ability to depolymerise variety of plastic polymers
- Defines and tests cocktail of enzymes that can be used at various stages of pre-treatment and after pre-treatment of mixed plastic waste
- Improves enzymes/biocatalysts using engineering approaches (directed evolution, immobilisation, etc.)
- Assess products of enzymatic depolymeration
- Supplies materials for valorisation



N/A.....

## ACADEMIC PARTNER PROFILE

### 3. Partner Profile Information

#### a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

The **University of Belgrade (UB)** is the oldest and the largest university in Serbia. Founded in 1808, at the moment it encompasses 31 schools, 11 research institutes, and 8 centres. **UB** is the largest learning community in this part of Europe, with 88 742 students (of which 4 124 international) and 3,736 academic and research staff and more than 300 study programmes. **IMGGE (www.imgge.bg.ac.rs)** was founded in 1986 as a Research Institute of UB specializing in **molecular genetics** and **biotechnology**. It employs 86 permanent researchers and 9 administrative and technical staff. It consists of 6 research laboratories that provide research facility and training for postgraduate students of the UB.

b) Relevant elements of the Curriculum Vitae/Biography (*including profile pictures*) of the team responsible for the project implementation. (*Please provide maximum 2 paragraphs per person and refer to the gender of each employee. Thank you*)

#### 1) Dr. Jasmina Nikodinovic-Runic – WP Leader; F (40% of full time planned)



ORCID 0000-0002-2553-977X

PhD in Molecular Genetics and Biochemistry, University of New South Wales, Sydney, Australia (2004)


Present position: Principal Research Scientist, LMMGE, IMGGE

Research experience: Conducted research in the field of molecular genetics of bacteria; Conducted research on directed evolution of various enzymes; Isolated and characterized novel biocatalysts, Worked on the conversion of petrochemical plastic monomers to PHA; Worked on bioprocess optimizations.

Research interest: **Microbial biotechnology** (bacterial fermentations, strain improvement, *Streptomyces* spp., *Pseudomonas* spp.); **biocatalysis** (protein expression & directed evolution, tautomerase, laccases, aminotransferases, biotransformations, biocatalytic process development and optimization); **Bacterial bioactive secondary metabolites** (biopigments, antifungal & anticancer compounds); **novel materials** (biopolymers, polyhydroxyalkanoates, functionalization of biopolymers)

Publications: 95

**2) Dr Lidija Djokic – F (30% of full time planned)**

 Associated with document Ref. Ares(2019)6080743 - 01/10/2019



ORCID 0000-0003-4723-0527

PhD in Molecular Biology, Faculty of Biology, University of Belgrade (2016)

Present position: Senior Research Associate

Research experience: Microbiology, Bioinformatics, Isolation of novel microbial strains, Screen and application of new biocatalysts.

Research interest: Biocatalysis, Enzymes, Biopolymers

Publications: 20

**3) Dr Sanja Jeremic – F (30% of full time planned)**



ORCID 0000-0002-6661-385X

PhD in Molecular Biology, Faculty of Biology, University of Belgrade (2013)

Present position: Research Associate, LMMGE, IMGGE

Research experience: Carried out metagenomic and culture-dependent analysis of microbial diversity in environment polluted with heavy metals, examined mechanisms of heavy metal tolerance and applied a defined bacterial consortium in bioleaching of contaminated environment. Studied biotechnologically relevant enzymes in *Pseudomonas* strains and recombinantly expressed and characterized laccases and lipases. Worked on designing of laboratory compost model system for polymer biodegradation validated on PCL-based polymers.


Research interest: Search for enzymes with potential for application in biotechnology and bioremediation, optimization of recombinant expression and enzyme characterization with aim of developing new biocatalysts. Use of biopolymers (nanocellulose) for biocatalyst immobilization.

Publications: 19

**4) Dr Jelena Radivojevic – F (40% of full time planned)**



0000-0003-3607-9649

 Associated with document Ref. Ares(2019)6080743 - 01/10/2019

PhD in Organic Chemistry, Faculty of Chemistry, University of Belgrade (2016)

Present position: Research Associate, LMMGE, IMGGE

Research experience: Synthesis and characterization of novel compounds; Synthesis of novel compounds using bacterial polymer PHA as starting material; Determination of antimicrobial and anti-proliferative activity of novel compounds; Research in the field of biocatalysis, worked on biocatalytic process development and optimization.

Research interest: Application of new biocatalysts in the synthesis of significant biologically active compounds. Research in the field of novel materials, (biopolymers, polyhydroxyalkanoates, etc) expanding application and finding new sources for their synthesis.

Publications: 6

#### 5) Dr Sandra Vojnovic– F (30% of full time planned)



0000-0002-5083-4287

PhD in Molecular Biology, Faculty of Biology, University of Belgrade (2011)

Present position: Senior Research Associate, LMMGE, IMGGE

Research experience: Conducted research in the field of molecular genetics of actinomycetes; Studied mRNA-protein interactions; Optimization of secondary metabolite production in soil actinomycetes by genetic manipulations; Bacterial fermentations

Research interest: Isolation and identification of bacterial secondary metabolites with antimicrobial activities; Bacterial strain improvements

Publications:23

#### 6) Dr Veselin Maslak – M (30% of full time planned)



ORCID 0000-0002-5735-3953

Associate Professor, Faculty of Chemistry-University of Belgrade

Research topics: organic synthesis, organocatalysis and biocatalysis, organic materials and supramolecular chemistry of fullerene; functionalization of bacterial polymers; PHA monomers as platform for derivatisations; biocatalysis.

Publications: 33

7) Dr Vladimir Beskoski –M (30% of full time planned)



ORCID 0000-0002-6372-4706

Associate Professor, Faculty of Chemistry-University of Belgrade

Research topics: Environmental biotechnology; microbial ecology; microbial activities and their applications in biogeotechnology and biohydrometallurgy; bioremediation; agriculture; application of the principles of green chemistry in the work with microorganisms; microbial consortium and changes in microbial diversity during bioremediation; elucidation of the genetic and biochemical basis of bacterial degradation of oil hydrocarbons and persistent organic pollutants, biotransformation of aromatic polymers such as lignin; products of microbial metabolism such as exopolysaccharides and rhamnolipids.

Publications: 41

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

1. Narancic, T., Djokic, L., Kenny, S.T., O'Connor, K.E., Radulovic, V., Vasiljevic, B., **Nikodinovic-Runic, J.** (2012) Metabolic versatility of Gram-positive microbial isolates from contaminated river sediments. *J Hazard Mater* 215-216:243-51
2. **Nikodinovic-Runic, J.**, Guzik, M., Kenny, S.T., Babu, R., Werker, A., O'Connor, K.E. Carbon-rich wastes as feedstocks for biodegradable polymer (polyhydroxyalkanoate) production using bacteria. *Adv Appl Microbiol* (2013) 84, 139-200
3. Guzik MW, Kenny ST, Duane GF, Casey E, Woods T, Babu RP, **Nikodinovic-Runic J**, Murray M, O'Connor KE. (2014) Conversion of post consumer polyethylene to the biodegradable polymer polyhydroxyalkanoate. *Appl Microbiol Biotechnol* 98(9):4223-32
4. Spasic J, Mandic M, Djokic L, **Nikodinovic-Runic J.** (2018) *Streptomyces* spp. in the biocatalysis toolbox. *Appl Microbiol Biotechnol* 102(8):3513-3536
5. O'Connor K.E., Gursky, L. and Nikodinovic, J. *Pseudomonas putida* styrene monooxygenase variants. WO2010003659 (A1)

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

1. 15th International Symposium on Biocatalysis and Biotransformations (BioTrans 2021)
2. The 17th **International Symposium on Biopolymers** (ISBP), 2020

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

**IMGGE** has specialised, fully equipped units: **1) PCR and sequencing unit** –3130 Genetic Analyzer, 7500 Real Time PCR System, 7900HT Fast Real Time PCR System (Applied Biosystems), NG sequencer – MiSeq(Illumina); 4-capillary sequencer; **2) Flow cytometry unit** – flow cytometer with cell sorter; **3) Microscopy unit** –confocal laser scanning microscope with *in vivo* imaging system, fluorescence microscope; phase contrast microscopes; **4) Chromatography unit** – HPLC, FPLC; **5) Zebrafish unit**; **6) Fully-equipped cell culture sterile rooms** for work with a) human/animal cell lines, b) pathogen bacteria, and c) plants. Standard equipment: RealTime-PCR equipment, centrifuges, ultracentrifuge, deep freezers -80°C, spectrophotometers, BioDoc analyser system, constant temperature rooms with shakers, electrophoresis systems (DGGE and 2-D), ultrasonic system, cryostat, biosafety level 2 flow laminar chambers, electroporation system, containers for liquid



nitrogen, autoclaves, safety cabinets for chemicals, etc. Through collaboration with the **FCUB** equipment for performing research in all branches of chemistry and biochemistry is available and includes: 500 MHz Bruker Avance III NMR spectrometer; 200 MHz Varian Gemini 2000 NMR spectrometer; atomic spectroscopy instrumentation (Inductively coupled plasma Thermo Scientific iCAP 6500 Duo, Varian SpectrAA 55 and Perkin-Elmer 2380 AAS Atomic Absorption); Agilent 7890A - 5975C Gas Chromatography-Mass Spectrometer System, comprehensive gas chromatograph-quadrupole mass spectrometer (GC×GC/MS) GCMS-QP2010 Ultra (Shimadzu, Kyoto, Japan) and GC-FID and GC-ECD (7890A Agilent, Santa Clara, California, USA) with headspace sampler (7697A Agilent, Santa Clara, California, USA); Elementar analyser Elementar Analysensysteme GmbH VARIO EL III CHNOS and Automatic polarimeter Rudolph Research Analytical Autopol IV

Additional instruments and apparatus for synthesis, purification and separation include: Gas Chromatography systems; Ion Chromatography System Dionex ICS 3000; Microwave digester Ethos 1 Milestone; TLC scanner Camag 3; Microwave reactor Microsynth Milestone, Flash chromatograph Biotage SP1, Controlled atmosphere glove box Protector, Labconco 50800-00 and the System for catalytic hydrogenation at low, medium and high pressures Parr Instrument Company. For measuring the metabolism rate in living organisms (simultaneous and continuous measurement of O<sub>2</sub>, CO<sub>2</sub>, CO, H<sub>2</sub>, NO<sub>2</sub>, H<sub>2</sub>S, CH<sub>4</sub>)-**Micro-Oxymax 12 Channel Computerized Respirometer** (Columbus Instruments, Columbus, Ohio, USA) with PC Workstation.

### PCR and sequencing:



### Microscopy



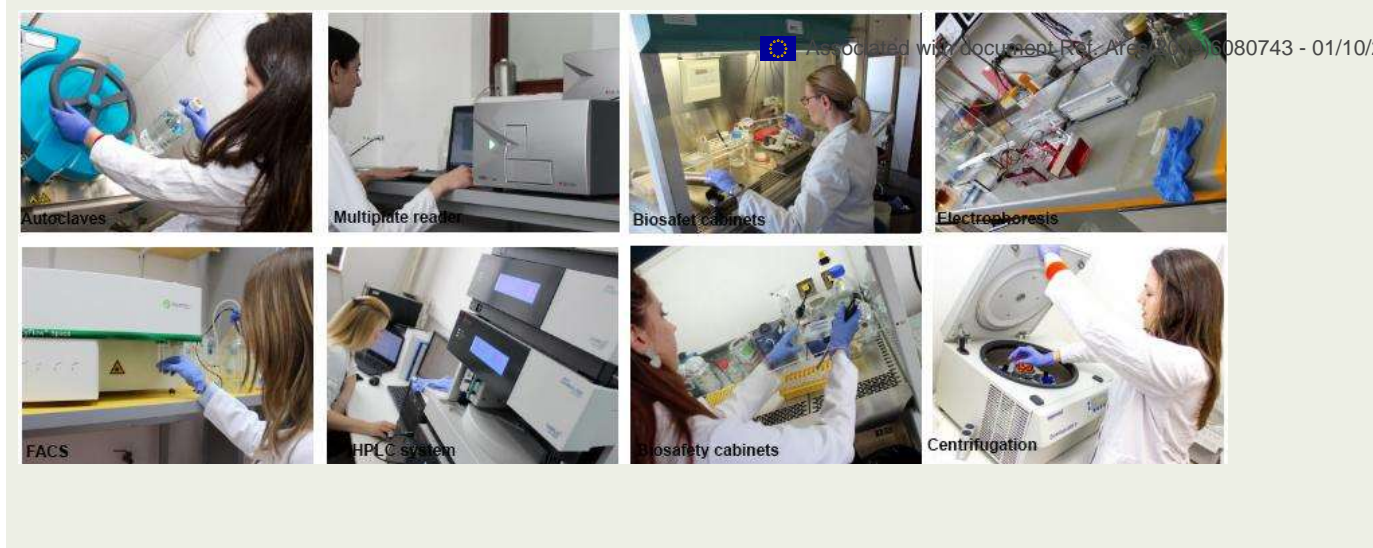
### Biobanks and bacterial cell collections



### Respirometer and fermenter



### Other equipment



## ACADEMIC PARTNER PROFILE

### Section 1. General Partner Information

1. Partner name: Instituto de Biologia Experimental e Tecnológica
2. Partner Website: <http://www.ibet.pt/>
3. Participant Identification Code (PIC) No: 999789865
4. Contact person name and email address: Prof. Maria Reis [amr@fct.unl.pt](mailto:amr@fct.unl.pt)
5. Position in organization: Senior Researcher
6. Department name: Industrial Bioengineering Lab
7. Average Person Month Rate in the organization: 3000€



## ACADEMIC PARTNER PROFILE

### Section 2. Technical Part Contribution

a) Which is the role of your organization in the proposal? *(Please explain in two sentences. Thank you.)*

To convert monomers from synthetic plastic (bio)degradation into biodegradable biopolymers and rhamnolipids using high fermentation yield strains. To produce biopolymers with different monomer composition (HB/HV) and chain length (scl-PHA and mcl-PHA). To produce nanocellulose with high quality. To produce rhamnolipids with different composition.

b) Which is the current state of the art of the technology you will introduce/progress during the project? Please provide a review of the recent scientific papers in the domain, patents and projects according to your knowledge. Thank you.

Production of PHA, nanocellulose and rhamnolipids using different microbial systems is well developed and implemented. Both monocultures and mixed microbial consortia have been used for the production of PHAs with distinct physical and chemical properties. A wide range of feedstocks has been demonstrated to be suitable to support cell growth, polymer synthesis and/or rhamnolipids synthesis. Examples include wastes such as glycerol from the biodiesel industry, used cooking oils, olive oil deodorizer distillate, margarine wastes, cheese whey, fruit pulp wastes, among many other

c) Does your team have recent scientific papers in this domain? (no more than 4 years) (Please provide a full list. Thank you)

1. Castro-Mayorga JL, Freitas F, Reis MAM, Prieto A, Lagaron JL (2018) Biosynthesis of silver nanoparticles and polyhydroxybutyrate nanocomposites of interest in antimicrobial applications. *Int J Biol Macromol* 108, 426–435

2. Cruz MV, Araújo D, Alves VD, Freitas F, Reis MAM (2016) Characterization of medium chain length polyhydroxyalkanoate produced from olive oil deodorizer distillate. *Int J Biol Macromol* 82:243-8
3. Pais J, Serafim LS, Freitas F, Reis MAM (2016) Conversion of cheese whey into poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by *Haloferax mediterranei*. *New Biotechnol* 33(1), 224-230
4. Pereira JR, Araujo D, Marques AC, Neves LA, Grandfils C, Sevrin C, Alves VD, Fortunato E, Reis MAM, Freitas F\* (2018) Demonstration of the adhesive properties of the medium-chain-length polyhydroxyalkanoate produced by *Pseudomonas chlororaphis* subsp. *aurantiaca* from glycerol. *Int J Biol Macromol* 122, 1144–1151
5. Cruz MV, Sarraguça MC, Freitas F, Lopes JA, Reis MAM (2015) Online monitoring of P(3HB) produced from used cooking oil with near-infrared spectroscopy. *J Biotechnol* 194, 1-9
6. Rui M C Portela, Moritz von Stosch, Rui Oliveira. Hybrid semiparametric systems for quantitative sequence-activity modeling of synthetic biological parts. *Synthetic Biology*, Volume 3, Issue 1, 1 January 2018,
7. Rui M. C. Portela†, Thomas Vogl, Claudia Kniely, Jasmin E. Fischer, Rui Oliveira, and Anton Glieder. Synthetic Core Promoters as Universal Parts for Fine-Tuning Expression in Different Yeast Species. *ACS Synth. Biol.*, 2017, 6 (3), pp 471–484
8. Moritz von Stosch, Rui Oliveira, Joana Peresa, Sebastião Feyo de Azevedo. Hybrid semi-parametric modeling in process systems engineering: Past, present and future. *Computers & Chemical Engineering*, Volume 60, 10 January 2014

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

European project: RES URBIS - H2020-CIRC-2016 OneStage – 730349: aims at making it possible to convert several types of urban bio-waste into valuable bio-based products, in an integrated single biowaste biorefinery and by using one main technology chain (2017-2019). Team role: production of PHA from urban bio-waste.

European project: EuroPHA - FP7-SME-2013-604770 : Turn agro-food waste into renewable packaging materials by biotechnological processes, to promote sustainable growth and contribute to the European Commission goal of a Bioeconomy (2013 - 2015). Team role: production of PHA from agro-food waste.

European project : YPACK - H2020-SFS-2017-1-773872 – High performance polyhydroxyalkanoates based packaging to minimize food waste (2017-2020) Team role: production of PHA from cheese-whey.

European project: NoAW - H2020-WASTE-2015-two-stage – 688338 (2016-2020) Team role: production of PHA from agricultural waste.

European project: INCOVER -H2020-WATER1b-2015- 689242 Innovative eco-technologies for resource recovery from wastewater” (2016-2019) Team role: production of PHA.

e) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*


Develop a process for production of PHA, nanocellulose and rhamnolipids, based on the use of waste plastic monomers as the sole feedstocks for microbial cultivation, some of which have never been tested before and, due to their complexity, may be difficult for microorganisms to assimilate them and convert into the biopolymers.

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- bioprocesses optimization for production of PHA, nanocellulose and rhamnolipids
- Processes validation
- Metabolic modelling and bioprocess optimisation, monitoring and control
- Development and optimization of downstream procedures for products' recovery

h) Which are the deliverables that your organization will deliver during the project?

- Report on the best strains and process operation conditions to produce the target products

- Report on the metabolic model and monitoring for process optimization  Associated with document Ref. Ares(2019)6080743 - 01/10/2019
- Report on the optimized conditions for downstream process

i) Which are the technical objectives that your organization needs to achieve during the project?

To develop a process for production of PHA, nanocellulose and rhamnolipids, based on the use of waste plastic monomers. ....

j) Please provide info regarding the market of the technology that your organization will develop (*size of market, main competitors, costs of services/products etc.* )

N/A.....

## ACADEMIC PARTNER PROFILE

### 3. Partner Profile Information

a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

iBET is an SME created to integrate and strengthen biological and biochemical knowledge from academic and industrial partners into technology for economic wealth and job creation. iBET brings together, as partners and collaborators, private companies and public institutions, creating a critical mass of competencies for product and process development. iBET comprises 16 laboratories and owns an adjacent bio pilot plant, in which companies have access to fermentation and downstream processing skills and equipment. iBET has coordinated over 20 international projects and has participated as work-package leader in more than 40 projects supported by the EC.

The iBET team has significant expertise on research in bioengineering, wastewater treatment, nutrient removal, microbiology and modelling, including the use of microbial based processes for biopolymer production (e.g. polyhydroxyalkanoates - PHA) and resource recovery from residues. Furthermore, this team has an extensive experience in training and supervision of higher education fellows (Masters, PhD and Post-Doc).

b) Relevant elements of the Curriculum Vitae/Biography (*including profile pictures*) of the team responsible for the project implementation. *(Please provide maximum 2 paragraphs per person and refer to the gender of each employee. Thank you)*



**Prof. Maria Reis** (female) iBET Researcher at Industrial Bioengineering Lab and FCT/UNL Professor. Her main research interests have been in the area of Environmental/Industrial BioEngineering, with special focus on the development of sustainable bioprocesses for the treatment and valorisation of industrial waste streams for the production of biopolymers and energy. Within this research area, she has published more than 200 papers in scientific journals with peer review and is co-author of 9 National/International Patents, and Coordinated 25 national and international projects (team leader), out of which 12 European Projects and 6 were co-funded by industrial companies, and participated as team member in 22 research projects.. She was a co-chair of the COST Action Water\_2020 (ES1202). She was elected as IWA Fellow, September 2010. She is Editor of the Water Research (Elsevier).





**Prof. Filomena Freitas** (female) iBET Senior Researcher at Industrial Bioengineering Lab. She has performed research on the development of upstream and downstream processes for the production of value-added microbial products, including polysaccharides and polyhydroxyalkanoates, as well as intellectual property development and technology transfer. Special focus is also given on the biological valorization of agro-industrial wastes/by products, aiming at implementing sustainable bioprocesses. She has 56 published papers in international peer review journals, 10 book chapters and over 60 conference proceedings. Overall, she has over 1200 citations and an h-index of 19. She has also 5 International Patents, which have recently been granted in several countries. She has participated in several projects in collaboration with Industries, including Project Gluecork, a QREN project in co-promotion with the company Amorim & Irmãos, Portugal, and GlyceroPol, in collaboration with 73100 Lda, Portugal.



**Dr. Nídia Lourenço** (female) is a Senior Researcher at Industrial Bioengineering Lab. She has expertise in biological treatment of industrial wastewater, including the operation of sequencing batch reactors with flocculent and granular mixed microbial cultures. She has also expertise in the development of multivariate models for real-time bioreactor monitoring based on online spectroscopy (ultraviolet-visible, near-infrared and Raman) and chemometrics. Her publication record includes 25 peer-reviewed papers with over 1100 citations and she has participated in 13 national and international research projects.



**Dr. Cristiana Torres** (female) is a post-doc researcher at Industrial Bioengineering Lab. Her research focus is on fermentation processes for production of value-added biopolymers (polysaccharides and polyhydroxyalkanoates) and fine chemicals (aromatic compounds), as well as the downstream process (purification steps) and physical-chemical characterization (chemical composition, rheology). She has 19 published papers in international peer review journals. Overall, she has 500 citations and an h-index of 11



**Prof. Rui Oliveira** (male) iBET Researcher at the Systems Biology Lab (head) and Associate Professor at FCT/UNL. His main research interests are in the field of Computational Systems Biology of single cells and mixed microbial consortia with special focus on hybrid systems methodologies for bioprocess optimisation, monitoring and on-line control. Within this research area, he has published more than 100 papers in scientific journals with peer review and is co-author of 6 National/International Patents, and Coordinated 10 national and international projects (team leader). He has been also deeply involved in innovation, where he has founded several start-up companies operating in the Systems Biotechnology arena.

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

9. Castro-Mayorga JL, Freitas F, Reis MAM, Prieto A, Lagaron JL (2018) Biosynthesis of silver nanoparticles and polyhydroxybutyrate nanocomposites of interest in antimicrobial applications. *Int J Biol Macromol* 108, 426–435
10. Cruz MV, Araújo D, Alves VD, Freitas F, Reis MAM (2016) Characterization of medium chain length polyhydroxyalkanoate produced from olive oil deodorizer distillate. *Int J Biol Macromol* 82:243-8
11. Pais J, Serafim LS, Freitas F, Reis MAM (2016) Conversion of cheese whey into poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by *Haloferax mediterranei*. *New Biotechnol* 33(1), 224-230
12. Pereira JR, Araujo D, Marques AC, Neves LA, Grandfils C, Sevrin C, Alves VD, Fortunato E, Reis MAM, Freitas F (2018) Demonstration of the adhesive properties of the medium-chain-length polyhydroxyalkanoate produced by *Pseudomonas chlororaphis* subsp. *aurantiaca* from glycerol. *Int J Biol Macromol* 122, 1144–1151
13. Cruz MV, Sarraguça MC, Freitas F, Lopes JA, Reis MAM (2015) Online monitoring of P(3HB) produced from used cooking oil with near-infrared spectroscopy. *J Biotechnol* 194, 1-9

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

1. European Symposium on Biopolymers (ESBP)
2. International Symposium on Biopolymers (ISBP)
3. International Conference on Biobased and Biodegradable Polymers
4. ....

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

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- 3 laboratories equipped with several lab scale reactors and analytical equipment
- FT-NIR and Raman spectrometers with optical fibre probes
- One Pilot plant (three 100 L bioreactors) for biopolymer production
- Zeiss Imager D2 epifluorescence microscope
- High speed tubular centrifuge (capacity 60-200L/h)
- Laminar flow cabinet
- Flow injection analyser for analysis of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$
- Complementary equipment for sample preparation (e.g. digesters, ovens, incubators, etc.)
- 3 GC with FID and TCD detectors.
- 5 HPLC with UV, RI, diode array detector
- TOC analyser

## ACADEMIC PARTNER PROFILE

### Section 1. General Partner Information

1. Partner name: **Limerick Institute of Technology**
2. Partner Website: <http://lit.ie/default.aspx>
3. Participant Identification Code (PIC) No: **990287939**
4. Contact person name and email address: **Dr. Patrick Murray**; [Patrick.Murray@lit.ie](mailto:Patrick.Murray@lit.ie)
5. Position in organization: Head of Research and Technology Transfer
6. Department name: Research Development and Innovation
7. Average Person Month Rate in the organization: Not available



### Section 2. Technical Part Contribution

a) Which is the role of your organization in the proposal? (*Explain in 2 sentences. Thank you.*)

We are contributing as WP leader and research provider for WP3 on the “Establishment of a catalogue of high performance microbial strains for plastic degradation and bioplastic production”

b) Which is the current state of the art of the technology you will introduce/progress during the project? *Please provide an overview of commercial solutions and initiatives in scientific community in the domain, patents and projects according to your knowledge*

During this project we will screen our biobank fungal strains and will isolate new bacterial and fungal strains from plastic contaminated field samples by using standard microbiological protocols and also by adopting state of the art new isolation technology. We will also utilize our experience on enzyme characterization as to identify the best possible microbial strains for plastic biodegradation and the producers of bioplastics through fermentation.

c) Does your team have recent relevant scientific papers in this domain? (no more than 4 years old) Please provide a full list

- Exploitation of Microalgae Species for Nutraceutical Purposes: Cultivation Aspects. Fermentation 4(2). 2018. Sushanta Kumar Saha & Patrick G Murray.
- Identification and manipulation of the pleuromutilin gene cluster from *Clitopilus passeckerianus* for increased rapid antibiotic production. 2016. Scientific Reports volume 6, Article number: 25202. Andy M. Bailey, Fabrizio Alberti,



- Improved method for rapid detection of phthalates in bottled water by gas chromatography–mass spectrometry Journal of Chromatography B 2015 v.997 pp. 229-235. 2015. Otero, Paz, Saha, Sushanta Kumar, Moane, Siobhan, Barron, John, Clancy, Gerard, Murray, Patrick.
- Sustainable production of biologically active molecules of marine based origin. New Biotechnology. 30:839-850. 2013. Murray, P., Moane, S., Collins, C. et al.
- Cloning, Heterologous Expression, and Characterization of the Xylitol and l-Arabitol Dehydrogenase Genes, Texdh and Telad, from the Thermophilic Fungus Talaromyces emersonii. 2010 Biochemical Genetics 48(5-6):480-95. Sara Fernandes, Maria Tuohy, Patrick G Murray.
- Xylose reductase from the thermophilic fungus Talaromyces emersonii: Cloning and heterologous expression of the native gene (Texr) and a double mutant (TexrK271R+N273D) with altered coenzyme specificity 2009 Journal of Biosciences 34(6):881-90. Sara Fernandes, Maria Tuohy, Patrick G Murray

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

- **LIT and Irish Bioeconomy Foundation** awarded €4.6 Million by Enterprise Ireland through the Regional Enterprise Development Fund. The purpose of the Irish Bioeconomy Foundation (IBF) is to promote the conversion of Ireland's natural resources on land and in the sea to high value products for the development of a sustainable Irish bio-economy that is globally competitive. The IBF propose to develop a National Bioeconomy Innovation & Piloting Facility for scale-up of processes to convert biobased resources to high value products at the Lisheen Mine site just outside Thurles. The proposed national facility would encompass a flexible, modular, pilot-scale multi-purpose chemical & biological infrastructure that would act as a test bed and be a driver for the scale-up of technologies from industry, universities and other research performing organisations enabling agri, food and marine companies to valorise their side-streams and residues to high value products. These value-added products will ultimately be consumed in a variety of industries including Nutraceuticals, biobased Chemicals, Pharmaceuticals, polymers etc. The facility will be a lynchpin which connects vitally important, but currently disparate elements in a burgeoning Bioeconomy ecosystem across Ireland. In doing so, it will act as a catalyst for the regeneration and re-industrialisation of Lisheen and the surrounding rural region by facilitating the efficient and cost-effective scale up and valorisation of new processes and technologies relevant to the bioeconomy in Ireland. It will enable diversification of business activities in Agri-food and marine sectors in the rural economy which will attract and retain workers and businesses in the region which in turn will drive innovation and investment. It will do so by acting as a "centre of gravity" for industry, entrepreneurs, academics, and ancillary service providers to interact, innovate and create new technologies, processes, products, companies and jobs.
- **European Commission. FP7 (265896).** Sustainable production of Biologically Active Molecules of Marine Based Origin (BAMMBO). 3 years €3 Million: BAMMBO screened and identified target marine organisms from diverse global locations for their potential to serve as sustainable producers of high value added bio-molecules (HVABs). Innovative solutions to overcome bottlenecks associated with developing economically sustainable, environmentally friendly and scalable culturing methodologies designed to produce high yields of value added products from marine resources for the pharmaceutical, cosmetic and industrial sectors were created. Novel analytical methods for the extraction, purification and enrichment of targeted bioactive compounds were developed. Life cycle analysis of the production pathways were undertaken to attain an environmentally holistic perspective of the sustainable production potential of HVABs from marine organisms. Knowledge and technology developed during the project in the form of know-how, new discoveries and novel inventions were documented as foreground IP and are progressing through the stages of IP protection. Non-IP sensitive outcomes were widely disseminated to target groups including researchers, policymakers and industrial stakeholders via a wide range of media routes as well as BAMMBO coordinated activities and participation at BAMMBO attended events. The European Strategy for Marine and Maritime Research encouraged capacity-building and promoted integration and synergies across all marine sectors. In addressing this funding received for BAMMBO and during ongoing BAMMBO research has enabled involved RTD and SME partner's participants to build their research and production teams and further facilitated the mobilisation of project participants to exchange technical experience and knowledge. BAMMBO's outcomes will serve in part to increase the competitiveness of the EU economy based on the capacity to create high value added knowledge based goods and services and foster the sustainable economic development of the marine sector.
- **Enterprise Ireland Innovation Partnership** 20130439 Company Partner Reagacon Ltd Shannon Ireland. New product development of testing standards for the food industry 2013 Duration (2 Years) €241,340. The development of a

- Limerick Institute of Technology **fungal and marine microbe biobanks** will be a valuable source of novel underexplored microorganisms for biodiscovery of enzyme systems relevant to plastic degradation and potential reutilisation.
- **Food Institutional Research Measure (FIRM) Dept of Agriculture, Food and the Marine** Mushrooms and Fungi, Functional and Life Enhancing Reservoirs FIRM 13 F 418 2013 (Duration 2 Years) [€162,139. Development of an Irish mushroom species specific bioactive profile relevant to the food and nutraceutical sector.

e) Does your company provide related services or products as the ones mentioned in the project? *Please provide more information regarding the content and the clientele receiving this kind of services/products. Thank you.*

**Limerick Institute of Technology (LIT)** is a third level Institute based in Ireland. The Institute's flagship research centre "Shannon Applied Biotechnology Centre" (Shannon ABC) was established through core funding provided by Enterprise Ireland under the Applied Research Enhancement (ARE) scheme. Shannon ABC has an ongoing programme to **explore natural organisms, natural products** for novel bioactive substances of **value to wide industry sectors**. LIT is currently coordinating, or participating in, a number of different European funded projects in the fields of science, renewable energy and sustainability, rural development and education. LIT's research strategy is the ambition to be a **strategic knowledge generator and technology transfer** partner of choice for companies and organisations facing the challenges of achieving sustainable competitive advantage in their marketplace. In line with the EU's research and innovation programme, Horizon 2020, LIT focuses on research excellence which **impact on industrial leadership and societal challenges**. We are recognized as a leader for educating high-achieving, motivated postgraduate research students who seek an individualized and transformative experience at an institution that generates and transfers knowledge through high-quality research partnerships, scholarships and creative activities.

f) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

Main challenges of BioICEP are identification of novel, efficient microorganisms with appropriate enzyme system for plastic biodegradation and bioplastic production. Our goal is to explore our existing terrestrial and marine biobank and also explore the new biobank strains, and identify the best strain or strains capable of plastic biodegradation and possibly recycle the degraded ingredients into bioplastics.

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

**T3.1. Biodiscovery screen of (existing and new) microbial biobanks:** Following the standard technique for screening of plastic-degrading microorganisms, which results in clear-halo zone on agar (**high-grade agar with reduced alternate carbon source or agarose**) plates, will be used by all partners for identifying plastic-degrading potential of their biobank strains. Selected recalcitrant plastic substrates (PET, LDPE, Polystyrene, HDPE and PU) and bio-based polymer substrates will be used either emulsified or dispersed in the medium as a fine powder as sole carbon source as described earlier (Deepika and Jaya Madhuri, 2015; Mao et al., 2015; Penkhrue et al., 2015; Skariyachan et al., 2017; Tang et al., 2017)

**T3.2. Isolation of new microbes and biobank enrichment:** Samples from waste plastics from landfills and marine habitats will be collected and bring to the laboratory for isolation using conventional plating technique. Biological material adhered to the plastic substrates will be removed by gentle scrapping, vortex and/or mild-sonication as appropriate in sterile water (10 ml). After serial dilution ( $10^{-5}$ ) of each samples, last three dilutions will be plated on nutrient agar for isolation of bacterial and fungal strains (Brunner et al., 2018). If some field samples are coloured particularly greenish/blackish, will be plated on modified BB/BG-11 (for fresh water) and/or ASN-III (for marine) agar medium for the isolation of photosynthetic microalgae/cyanobacteria (Otero et al., 2017). Following the iCHIP method using an empty rack from the Matrix TallTip Extended Length Pipette Tip box (Berdy et al., 2017), we will isolate the bacterial and fungal strains on neutral agar with standard mixed plastics or nutrient agar. Last three dilutions as above of each samples will be mixed with molten agar for the

diffusion chambers in iCHIP and sealed with 0.03- $\mu$ m-pore-size polycarbonate membranes to each side of the rack, and then incubate the iCHIPs to the corresponding natural habitat for 1-3 months before final laboratory isolation.

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**T3.3. Liquid media cultivation with standard plastics:** Cultivate the pre-identified plastic degrading organisms from **T3.1** and **T3.2** in appropriate minimal media with standard plastics (single and in mixes). This test will be performed in flasks containing minimal media and the plastics and inoculated with selected microbes from above tasks. Following growth period, the culture supernatant (cell-free extracts) and spent medium (source for extracellular enzymes) will be collected and tested for depolymerase enzyme activities. The plastics after microbial degradation will be collected and analysed as part of **T3.5**.

**T3.4. Liquid media cultivation with pre-treated plastics:** Test the identified plastic degraders from liquid cultivation in **T3.3** for their ability to degrade targeted plastics which have been pre-treated as part of WP2. This test will be performed in flasks containing minimal media containing pre-treated plastic and inoculated with selected microbes from above **T3.3**. Following growth period, the culture supernatant (cell-free extracts) and spent medium (source for extracellular enzymes) will be collected and tested for depolymerase enzyme activities. The plastic after microbial degradation will be collected and analysed as part of **T3.5**.

**T3.5. Quantitative/qualitative analysis of plastic breakdown potential and dynamics:** Plastic subjected to microbial degradation in T3.3 and T3.4 will be analysed in this task. The reduction in weight of biologically treated plastics will be recorded by digital balance which could accurately measure up to 0.01 mg. The thickness of plastics will be measured using digital micrometre capable of measuring up to 0.001 mm. Weight loss efficiency will be calculated using following equation,

$$\text{Weight reduction (\%)} = [(W_0 - W_t) \times 100] / W_0$$

Where,  $W_0$  is the initial weight of plastic (g),  $W_t$  is the weight of plastic (g) at time 't' (days). Rate constants for plastic degradation will be determined using the relation given below:

$$-\ln (W_t/W_0) = kt$$

Where  $W_0$  is the initial weight of plastic (g),  $W_t$  is the weight of plastic (g) at time 't' (days) after microbial inoculation. A plot of  $\ln W_t/W_0$  versus 't' yields a slope equal to 'k' which is rate constant.

The chemical fingerprint of the biodegraded plastics will be assessed by attenuated total reflectance (ATR)-FTIR spectroscopy used at a frequency range of 4500–400  $\text{cm}^{-1}$  for analysis. To investigate the chemical modifications of the plastics induced by the microbial growth, the IR spectra of the plastics before and after inoculation with microbes will be compared. For these analyses, 10 randomly chosen spots will be assayed in three replicates of treated and untreated plastic.

**T3.6. Identification of potential PHB, rhamnolipid and nanocellulose producers:** Screen pre-identified degraders from **T3.3** and **T3.4** as potential producers for PHB, rhamnolipid and/or nanocellulose production after cultivating these strains on **breakdown plastic products** (styrene, terephthalic acid, some isocyanates, ethylene glycol, polyol, lactic acid, 3-hydroxybutyrate, 3-hydroxyoctanoate, glucose) (in broth or on plates as appropriate). Nile red or Nile blue A staining plate assay method will be used for the identification of **PHA/PHB producers**. In brief, plates will be prepared with neutral agar, breakdown plastic products and Nile red or Nile blue A, and after incubation with the test organism, plates will be exposed to ultraviolet light (312 nm) to visualise stained intracellular **PHA/PHB** granules (Spieckermann et al., 1999). PHB production and content will further be confirmed through liquid cultivation of the suspected positive organisms. **Epifluorescence microscopy** using Nile-red staining for rapid identification of short-chain-length and medium-chain-length PHBs will be used as described earlier (Wu et al., 2003). From liquid cultivation, 5-15 mg dried biomass will be used for **GC-MS analysis** for precise information on the content and type of PHB produced (de Rijk et al., 2005). Blue agar plate assay method will be used for the identification of **rhamnolipid producers**. In brief, plates will be prepared with neutral agar, breakdown plastic products, cetyl-trimethyl-ammonium bromide and methylene blue, and after incubation with the test organism, appearance of dark blue halo zone around the culture will be considered positive for rhamnolipids (Pinzon and Ju, 2009). Buffered Schramm & Hestrin's (BSH) agar plate assay method will be used for the identification of **nanocellulose producers**. In brief, pre-identified degraders will be cultured on BSH medium (**carbon source replaced with breakdown plastic products**) statically for 2 weeks. Culture broth showing pellicles formation will then be spread onto BSH agar plates and incubated again for a week. The colonies with milk-white and swollen appearance will be isolated as potential **nanocellulose producers** (Naritomi et al., 1995; Jeremic et al., 2019).

**T3.7. Material Transfer Agreement:** At the conception of this project (BioICEP) all partners should agree to sign the Material Transfer Agreement (MTA), which will allow the freedom to the partners related to **WP4**, **WP5** and **WP6** to use the strains

identified in **WP3**. This agreement will be updated during sharing the strains by each partners according to KTI (Knowledge Transfer Ireland) or other standard protocols followed by each partner Institute/organisation. All details relating to IP issues will be properly addressed in a **consortium agreement** (CA), which will be finalized during the contract negotiations. This agreement will provide the legal framework within which all partners can work freely, thus maximizing opportunities for effective collaboration and exploitation. IP rights of the consortium partners will be respected in all events.

**T5.1:** Forming synthetic communities using established plastic degrading strains. After determining optimal plastic breakdown potential of existing strains and communities and of newly discovered communities from **WP3** synthetic communities will be formed.

**T5.2:** Complementary to task 3.1 we will enrich relevant, natural communities to increase their plastic degradation potential. Relevant existing communities which contain plastic degrading microbes will probably contain these organisms at low densities. By enriching them on plastic model compounds (mostly plastic dimers) to be used as a carbon source the density of the plastic degrading microbes will be increased and species that do not contribute to the degradation process will be lost yielding a relevant and efficient degrading community. Communities already present at the project partners but also newly discovered communities from **WP3** will be subjected to enrichment processes using model compounds selected and tested in **WP4**. Subsequently these enriched communities will be tested on their breakdown potential of mixed plastic waste streams.

**T5.5:** Identification of plastic transformation and breakdown products from degradation by microbial consortia. After optimal breakdown consortia have been established in previous tasks, we will investigate which breakdown products are generated from the selected types of plastic and how these breakdown products can feed into subsequent fermentation processes to generate new products (WP6). Examples of breakdown products can be plastic monomers such as terephthalic acid from PET or oligomers which consist of short chains of the respective plastic monomers. Ideally the selected breakdown processes would not mineralize plastics to CO<sub>2</sub> and H<sub>2</sub>O so the breakdown products can be used as a carbon source to ferment into new products. Plastic breakdown products will be analyzed using GC-MS, LC-MS.

h) Which are the deliverables that your organization will deliver during the project?

**D3.1** Isolation and establishment of new biobank microbial strains

**D3.2.** Identification of microbial degraders of plastics from existing and new biobanks

**D3.3** Establishment of liquid cultivation conditions for pre-identified degraders on standard and pre-treated plastics

**D3.4** Characterisation of depolymerase enzyme activities after liquid cultivation

**D3.5.** Quantitative and qualitative characterisation of plastic breakdown potential and dynamics

**D3.6.** Identification of PHB, rhamnolipids and nanocellulose producers

**D3.7.** Consolidated identification and recommendation of the best microbes and their growth conditions to support WP4, WP5 and WP6 for various up-scaling optimisations.

**D3.8.** Sharing of identified strains, their growth conditions for consortia optimisation after signing of appropriate Material Transfer Agreement (MTA)

**D5.2.** Information on synthetic community vs individual microbe performance for plastic breakdown based on combined data from **WP3** and **WP5**.

**D5.5.** Information on plastic breakdown products to be fed as carbon sources into the fermentation processes developed by **WP6**

i) Which are the technical objectives that your organization needs to achieve during the project?

As mentioned above in **T3.1-T3.7** and **T5.1, T5.2** and **T5.5**.

j) Please provide info regarding the market of the technology that your organization will develop. Thank you. (*size of market, main competitors, costs of services/products etc.*)

General information from

BioCEP .....

### 3. Partner Profile Information

#### a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

**Limerick Institute of Technology (LIT)** is a third level Institute based in Ireland. LIT is currently coordinating, or participating in, a number of different European funded projects in the fields of science, renewable energy and sustainability, rural development and education. LIT's research strategy is the ambition to be a strategic knowledge generator and technology transfer partner of choice for companies and organisations facing the challenges of achieving sustainable competitive advantage in their marketplace. In line with the EU's research and innovation programme, Horizon 2020, LIT focuses on research excellence which impact on industrial leadership and societal challenges. We are recognized as a leader for educating high-achieving, motivated postgraduate research students who seek an individualized and transformative experience at an institution that generates and transfers knowledge through high-quality research partnerships, scholarships and creative activities.

#### b) Relevant elements of the Curriculum Vitae/Biography *(including profile pictures)* of the team responsible for the project implementation *(Please provide maximum two paragraphs per person and refer to the gender of each employee. Thank you.)*

**Dr. Patrick Murray** is the Head of Research and Technology Transfer at Limerick Institute of Technology. Dr Murray



is also a Principal Investigator in the Shannon ABC Research group at LIT. Dr Murray previously held the position of research coordinator at Shannon ABC Centre and therefore he has a strong track record of collaboration with industry specifically in identification of innovative ingredients and biological products from natural resources using novel state of the art processes to obtain lead molecules for drug development and value added food, flavour and medicinal products. Dr. Murray was the Scientific Coordinator and WP4 leader of an EU FP7 project BAMMBO on extraction of high-value bioactive molecules from marine plants and animals with specific interests on environmentally friendly and sustainable extraction processes (using Supercritical Carbon Dioxide). The BAMMBO project was coordinated by LIT (2010 - 2013). Dr. Murray was the principal investigator of an Enterprise Ireland funded project in partnership with an Irish SME "AlgaeHealth" involving the scale-up of indoor cultivation in photobioreactor systems and extraction of high value bioactive molecules from microalgae. Dr. Murray is currently principal investigator for three Industrial Innovation partnership projects funded by Enterprise Ireland. Dr. Murray has also directed projects involved in bioconversion of target biomass streams to fermentable feedstock's for bioethanol/biodiesel production. Dr. Murray received his Degree in Biochemistry from the National University of Ireland, Galway (NUI Galway) and subsequently completed his PhD in Fungal Glycobiotechnology, also at NUI Galway. Dr. Murray developed the molecular biology laboratory as part of the Molecular Glycobiotechnology Group at NUI Galway, and has previously worked as a visiting scientist at Wallenberg Wood Biotechnology Centre and at VTT research centre in Finland. Dr Murray is also supervising postgraduate students towards their masters and PhD degrees. Dr. Murray is co-owner of 2 patents and the author to a number of peer reviewed scientific articles. Dr. Murray provides scientific leadership to the development of industrially relevant projects as well as technology transfer.

**Dr. Catherine Collins** obtained her PhD for investigating the molecular genetics of cellulose degradation in the cellulolytic fungus *Talaromyces emersonii* at the National University of Ireland, Galway. Since then she has worked on a number of fungal projects both in Ireland and the U.K. including a project at the University of Bristol, England, which was in collaboration with GlaxoSmithKline, involving cloning, sequencing and manipulating the genes involved in production of the antibiotic pleuromutilin from the mushroom *Clitopilus passeckerianus*. Currently she is employed as a Post-Doctoral Researcher at Shannon Applied Biotechnology Centre (SABC), Limerick Institute of Technology (LIT) for investigating the properties of peat based skin care products. Previously, Dr. Collins worked as a researcher on the FP7 EU funded project BAMMBO (Biologically Active Molecules of Marine Based Origin) at



SABC, LIT. In the above project marine organisms (fungi, yeast, bacteria, macro-algae, micro-algae and sponges) were screened for their potential as sustainable producers of high-added value molecules (HVABs). At Shannon ABC, LIT she is applying analytical methods for the extraction, purification and enrichment of bioactive compounds. She has just recently established her own research group which focuses on investigating mushrooms and fungi as functional foods and a source of bioactives and the project was funded by the Department of Agriculture, food and the marine [€162,139 for two years with two postgraduate researchers].



**Dr. Sushanta Kumar Saha** is currently working as Microalgal Biotechnologist at Shannon Applied Biotechnology Centre with twenty years of research experience on cyanobacteria and eight years' experience of microalgal biotechnology. At present, he is the PI and SRM of a Commercial Fund project funded by Enterprise Ireland on natural colourants from microalgae and cyanobacteria. He was instrumental in developing and was the principal researcher on an Innovation Partnership project with Reagecon (2013-2015) on microalgal bioactive production. He also contributed as a senior researcher on the EU FP7 -KBBE BAMMBO project. He was the principal senior researcher for another Innovation Partnership project with an Irish microalgal company. He successfully transferred the laboratory know-how for the industrial-scale cultivation of microalgae for the production of carotenoid astaxanthin to Algae Health. Here at Shannon ABC, he has established an Irish cyanobacterial biobank and other potential microalgae strains for evaluating specific value-added biomolecules for their most possible biotechnological applications. Earlier, he was involved in Department of Energy, USA and National Science Foundation, USA funded projects in the Department of Biology, Texas A&M University and Division of Biological Sciences, Molecular Biology, University of California, San Diego, USA, as a postdoctoral research associate for six years. He supervised two postgraduate students for their PhD and master's degree by research. He is at present supervising other students for their PhD and master's degree on microalgal biotechnology. He has vast knowledge on recent molecular biology, biochemistry and microbiological techniques and taught/demonstrated on recent Molecular Biology Techniques consecutively for four years to the participants of the "National Level Workshops on Cyanobacteria" conducted by National Facility for Marine Cyanobacteria, Bharathidasan University, India. He has published about twenty research and review articles including two invited book chapters, as main contributing author. He is also contributing as invited reviewer for more than thirty journals and also served as editorial board member for online free-access journals. He has contributed to the project report writing in all projects he was associated with, such as in the USA, India and Ireland. Dr. Saha is recipient of CHIMERA research Cluster BURSARY 2014 award to work on Evaluation of Irish Diatoms for Biofuel production. Dr. Saha is a Co-ordinator and PI for LIT funded Bursary Graduate Research projects. He has supported development of an IRCSET research proposal that was funded (GOIPG/2013/32, €72,000 for 3 years) for a PhD research student to work on "Evaluation of Irish marine cyanobacteria for sunscreen compounds: their growth optimisation and genetic improvement".

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

1. Exploitation of Microalgae Species for Nutraceutical Purposes: Cultivation Aspects. *Fermentation* 4(2). 2018. Sushanta Kumar Saha & Patrick G Murray.
2. Identification and manipulation of the pleuromutilin gene cluster from *Clitopilus passeckerianus* for increased rapid antibiotic production. 2016. *Scientific Reports* volume 6, Article number: 25202. Andy M. Bailey, Fabrizio Alberti, Sreedhar Kilaru, Catherine M. Collins, Kate de Mattos-Shiple, Amanda J. Hartley, Patrick Hayes, Alison Griffin, Colin M. Lazarus, Russell J. Cox, Christine L. Willis, Karen O'Dwyer, David W. Spence & Gary D. Foster
3. Improved method for rapid detection of phthalates in bottled water by gas chromatography–mass spectrometry *Journal of Chromatography B* 2015 v.997 pp. 229-235. 2015. Otero, Paz, Saha, Sushanta Kumar, Moane, Siobhan, Barron, John, Clancy, Gerard, Murray, Patrick.
4. Sustainable production of biologically active molecules of marine based origin. *New Biotechnology*. 30:839-850. 2013. Murray, P., Moane, S., Collins, C. et al.
5. Cloning, Heterologous Expression, and Characterization of the Xylitol and l-Arabitol Dehydrogenase Genes, *Texdh and Telad*, from the Thermophilic Fungus *Talaromyces emersonii*. 2010 *Biochemical Genetics* 48(5-6):480-95. Sara Fernandes, Maria Tuohy, Patrick G Murray.



6. Xylose reductase from the thermophilic fungus *Talaromyces emersonii*: Cloning and heterologous expression of the native gene (Texr) and a double mutant (TexrK271R+N273D) with altered coenzyme specificity 2009 Journal of Biosciences 34(6):881-90. Sara Fernandes, Maria Tuohy, Patrick G Murray.

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

**Possible journals for publications:**

1. Applied and Environmental Microbiology (ASM journal, USA; ISSN: 0099-2240)
2. Biodegradation (Springer Journal; ISSN: 0923-9820)
3. Molecular Biotechnology (Springer Journal; ISSN: 1073-6085)

**Dissemination:**

4. After protecting appropriate IP, the significant research outcomes will be disseminated at national & international conferences and via newspaper articles, LIT newsletters (Shannon ABC and RDI newsletters) and news media documentaries as appropriate, such as RTE news.

**Conference and trade shows**


5. 23<sup>rd</sup> European Biotechnology Congress (September 9-10, 2019 Zurich, Switzerland)
6. 6<sup>th</sup> World Congress and Expo on Applied Microbiology (24-25 October, 2019 Rome, Italy)

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

LIT has provided laboratory space of 100 m<sup>2</sup>P in its Hartnett Centre (incubation and research are co-located in this centre) to house Shannon Applied Biotechnology Centre (Shannon ABC) and its equipment ([www.shannonabc.ie](http://www.shannonabc.ie)). Adjacent to Shannon ABC is a 100 m<sup>2</sup>P Department of Applied Science Research Laboratory. In May 2008, the LIT secured matched funding of €500,000 from the Higher Education Authority Research Facilities Enhancement Scheme (HEA-RFES) for a €1.12 M development of this laboratory which includes €500,000 of analytical equipment (listed below). Subsequently, LIT also secured funding from the Enterprise Ireland: Research Equipment Grant in July 2008 (EI-REG) for the purchase and commissioning of growth chambers, from Environmental Growth Chambers (EGC), Ohio. These chambers are identical to those currently located in the Space Life Sciences Laboratory at Kennedy Space Centre, Florida where they are used to conduct ground based research on production of plants and microbes. A purpose built laboratory is in place for this project containing state-of-the-art equipment including specialised growth chambers and high-tech analytical equipment (listed below). Shannon ABC has an ongoing program to explore natural products for novel bioactive substances of value to a variety of industries. Natural products include under-explored and under-exploited materials from marine microalgal sources.

- Environmental Growth Chambers (2x Walk-In + 2x Reach-In)
- Fungal microbial biobank
- Algal/cyanobacterial microbial biobank
- Class II microbiology containment laboratory
- Supercritical Fluid Extraction System
- LC/MS-MS
- GC/MS-MS (EI & CI)
- GCFID/TCD
- AAS-GF
- HPLC -Rapid Resolution
- Freeze Dryer
- UFMF
- Monochromatic Microtitre Plate Readers with Fluorescence & UV -Vis detection

- Bench top Automated Multiple Pipetting System
- Air Monitoring System
- Ultra Violet/Visible Double Beam Spectrometer
- Ultra Violet/Visible Double Beam Spectrometer (Enzyme Kinetics)
- Protein Purification System
- Capillary Electrophoresis
- Gel Electrophoresis System (including Gel Doc Unit)
- Solid Phase Extraction Manifold
- FTIR with Attenuated Total Reflectance

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## INDUSTRY PARTNER PROFILE

### Section 1. General Partner Information

8. Partner name: Logoplaste Innovation Lab
9. Partner Website: [www.logoplaste.com](http://www.logoplaste.com)
10. Participant Identification Code (PIC) No: 966190423
11. Contact person name and email address: Maria Eugenia Zacarias,
12. Position in organization: Raw Materials, Sustainability and Legislation Manager
13. Department name: Raw Materials, Sustainability and Legislation
14. Average Person Month Rate in the organization: 6.108,83€



## INDUSTRY PARTNER PROFILE

### Section 2. Technical Part Contribution

a) Which is the role of your organization in the proposal? *(Explain in 2 sentences. Thank you.)*

The role of Logoplaste Innovation Lab is to assure the integration of the Bio-material produced by the Consortium from waste stream using Biotechnology, into food contact materials by the production of containers by Extrusion Blow Molding Technology. Conceptual design, CAD design, Finite Element Analyses, Quality Assurance tests and prototyping will be developed.

b) Which is the current state of the art of the technology you will introduce/progress during the project? *Please provide an overview of commercial solutions and initiatives in scientific community in the domain, patents and projects according to your knowledge*

In the current technological state, industrial machines for rigid packaging production are optimized to process very specific fuel-based materials, such as PET, HDPE or PP. Therefore, processing settings, controls and monitoring equipment are also designed to that end, which imply a lack of knowledge on how to properly design the industrial process settings for bio-based materials, or even to design a product that is able to accommodate its restrictions.

c) Does your team have recent relevant scientific papers in this domain? (no more than 4 years old) Please provide a full list

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

- FP7-NMP-2011-SMALL-5 – PHBOTTLE . New sustainable, functionalized and competitive PHB material based in fruit by-products getting advances solutions for packaging and non-packaging applications. Logoplaste Innovation Lab Support the customization of the different formulations of the PHB material produced from the waste stream and develop all trials for the validation of the existing technologies used with thermoplastic materials (EBM and ISBM) with the aim to customize process parameters together with the indicated geometry to produce a container for juice.
- FP7-NMP-2011-Large5 NanoBarrier – Extended Shelf-life biopolymers for sustainable and multifunctional food packaging solutions for Europe by integrating innovative nanotechnology-based barrier promoters and sensor materials with biomaterial formulations. Logoplaste Innovation Lab contributed with the support regarding the selection of the most indicated materials coming from renewable resources and validating its barrier performance for oxygen sensitive products and its use for the production of containers for sauces or dressing by Injection Stretch Blow Molding
- H2020-ICT-03-2014 – Roll-Out .Advanced Thin, Organic and Large Area Electronics (TOLAE) technologies. Logoplaste Innovation Lab contributed with the identification of the functionalities desired to produce a Smart Packaging, developing an innovative sport bottle with customized features to integrate the respective sensors, circuits and batteries developed by Roll-Out Technology.

e) Does your company provide related services or products as the ones mentioned in the project? *Please provide more information regarding the content and the clientele receiving this kind of services/products. Thank you.*

Logoplaste Innovation Lab (ILAB) is dedicated to the development of high performance rigid plastic packaging solutions, which includes all activities from Design, CAD modelling, FEA modelling to the production phase on laboratory and industrial scale machinery and quality assessment. Logoplaste Innovation Lab develops its work for large FMCG companies such as P&G, Kraft-Heinz, Unilever, SCJohnson, Suntory, Danone, among others.

f) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

For every raw material used in the production of a bottle, there is a set of process conditions to be respected in order to obtain the envisioned result. Moreover, the finished product must respect a set of specifications, namely dimension, mechanical resistance and consumer safety, especially in the case of food contact packaging. Making use of its core skillset, ILAB will therefore act as the developing partner for the packaging solution, employing efforts to make either the bottle and the manufacturing process feasible according to project and market specifications.

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- Task 7.9 – Packaging Design:  
Different design of experiments will be planned during the project life to achieve the optimal parameters for

each compound iteration developed in WP6 or WP7. The packaging design will undergo development having in consideration the different requirements in terms of functionality and safety, either to human health and the environment. Each variant of the processing technology used in this WP – Extrusion Blow Molding – has its own characteristics in terms of process conditions, and the biomaterial will need to be formulated to achieve the desired rheological properties, which will limit the packaging geometry and design.

Once the biopolymer compounds have been characterized in WP6, CAE simulations (processing, mechanical properties and transport) will be carried out in order to previously identify the behaviour of the material in terms of processing, with the objective to verify the required mechanical properties.

- Task 7.10 – Processability studies of PHA compound(s)
- Subtask 7.10.1: Processability in standard monolayer blow-moulding equipment.

PHA materials will be used to produce a bottle with a capacity between 250ml and 350ml by Extrusion Blow Molding in a continuous process of a TECHNE machine Module 2000 available at ILAB. In this task all process conditions like Temperature profile, Blowing Pressure, Cycle time (output), Parison wall thickness profile will be registered and monitored for further analysis. Packaging performance evaluation will include: dimensional analysis, weight, wall thickness distribution and top load. Aiming to improve packaging performance: bottle geometry will be evaluated and modified if necessary; process parameters will be adjusted to improve material distribution in the weak areas of the bottle and bottle weight will be

adjusted to match current bottle specification. To improve bottle visual appearance, extrusion tools (Pin and Die) will be evaluated and if necessary redesign, re-polished or covered with a surface treatment aiming to reduce material friction.



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#### Subtask 7.10.2: Processability in multilayer blow-molding equipment.

This step will be used by ILAB to test the usage of the PHA layer as an inner layer of a 3 layer bottle. The same bottle will be produced in the BEKUM BM 304SM through a multilayer extrusion blow molding continuous process. To adapt to this process, the mold will be evaluated and redesigned if necessary. Inner layer percentage must be defined and refined throughout the process. As it was done with monolayer, process conditions (Temperature, Blowing Pressure, Cycle time, Parison wall thickness) will be registered and monitored for further analysis. Performance evaluation will be evaluated with the same metrics: dimensional analysis, weight, wall thickness distribution and top load. To improve packaging performance, same iterative process will be applied (almost): bottle geometry will be evaluated and modified if necessary; process parameters will be adjusted to improve material distribution in the weak areas of the bottle, bottle weight and percentage of inner layer will be adjusted to match current bottle specification.

h) Which are the deliverables that your organization will deliver during the project?

- Identification of requirements. Bottle design and technical drawings;
- FEA Simulations Report;
- Optimized process window for all use cases;
- Packaging Characterization Report.

i) Which are the technical objectives that your organization needs to achieve during the project?

The technical objectives are: the design of a packaging that is suitable to incorporate the materials under development and, at the same time, fulfill the product specifications; design a manufacturing process specification that enables a feasible use of the material, validating it as an alternative to standard fossil-based materials.

j) Please provide info regarding the market of the technology that your organization will develop. Thank you. (*size of market, main competitors, costs of services/products etc.*)

Bioplastics still represents a small fraction of the global plastics production (about 1%). Within that small percentage, 65% are used towards packaging solutions and the PHA represents only 1,4% of global bioplastics production. The price of purchase for a raw materials such as PHA is 3-4 times higher than conventional polymers, which illustrates the need for improvements in the manufacturing process or alternative production technologies with reduced costs will be an advantage. Main producers of these raw materials are BioMatera, Biomer, Bio-on, Danimer Scientific, TianAn Biologic Materials, Tianjin GreenBio Materials and Yield10 Bioscience.

Main applications of PHA are related with: single use packaging for foods and beverages; medical appliances, such as sutures or bone plates; agricultural foils and films. Today it use in rigid packaging is still dedicated to niche applications and also compounding of the materials are required to achieve the requirements of the processing technologies used today in thermoplastics.

## INDUSTRY PARTNER PROFILE

### 3. Partner Profile Information

a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

Logoplaste is an industrial group, manufacturing rigid plastic packaging for some of the most reputable companies in the world, in the food and beverage, personal care, household care and oil and lubricants sectors.

Founded in 1976, for over 40 years, the company has pioneered in-house manufacturing in Europe and beyond with the "hole in the wall" concept, supplying plastic bottles "just-in-time" from factories installed directly on the site of the client.

Today, ILAB manages more than 60 factories, more than 350 machines, with locations in 16 countries: Brazil, Belgium, Canada, Czech Republic, France, Italy, Mexico, Netherlands, Poland, Portugal, Russia, Spain, Ukraine, United Kingdom, USA and Vietnam. The most up-to-date technologies in injection molding, stretch-blow molding and extrusion molding are used to produce packages across the wide range of market segments.

Logoplaste Innovation Lab (ILAB) is an independent business unit of the Logoplaste Group that was founded in 2000 and is actively dedicated to the research and development of high performance plastic packaging solutions.

ILAB plans, organizes, secures, and manages resources to meet specific goals that bring beneficial change or added value, engaging team to deliver the best packaging solutions to our partners.

ILAB provides Sustainable Human Centered Innovation, applying creativity to the formulation and resolution of challenges, delivering the most desirable, feasible and viable solutions.

ILAB is engaged in Innovation inspired by Nature, using it as a model, a measure and a mentor to create more sustainable designs. This shift from learning about nature to learning from nature requires a new method of inquiry, a new set of lenses, and above all, a new humility.

ILAB continuously searches for the best, safest and most sustainable raw materials to improve packaging and its manufacturing processes.

ILAB is capable of producing quick physical prototype models, allowing our designers and engineers to explore and test more iterations, catching potential flaws before incurring in higher costs of re-tooling and rework, leading to better informed product decisions on time and budget.

ILAB has all the lab's resources to produce and validate samples, and define processes for plastic packaging using IM, SBM and EBM technologies, ensuring faster time to market solutions with optimized costs.

ILAB offers a wide range of standard and tailor made tests, analyzes capabilities of plastic based packaging and processes, and defines product specifications, innovating on new systematic approaches and scoring methods.

b) Relevant elements of the Curriculum Vitae/Biography (*including profile pictures*) of the team responsible for the project implementation (*Please provide maximum two paragraphs per person and refer to the gender of each employee. Thank you.*)



**Paulo Correia:** R&D Director of the Group LOGOPLASTE, and responsible for the business Unit

LOGOPLASTE Innovation Lab.



**Maria Eugenia B. Zacarias:** Polymer Engineering degree of the Simon Bolivar University

(Venezuela) with a Post-graduation in Food Safety at the Portuguese Catholic University.

Accumulated expertise in the packaging area since 1988 and since 2000 responsible at ILAB by the

R&D activities of Raw Materials, Sustainability and Regulatory Affairs

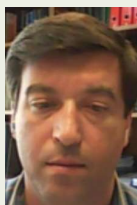


**Verónica Salgueiro:** Chemical Engineer. Work experience in food contact plastic rigid packaging

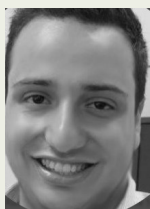
plants Quality Assurance Systems. Currently working in Customer Support on Product and Mould design for plastic rigid packaging, working as Project Manager at Logoplaste Innovation Lab since 2007.



**Bruno Machado:** Polymer Engineer. With previous work experience in Industrial Research, has been conducting project management activities within Logoplaste for 12+ years, now sitting as Technical Director of Logoplaste Innovation Lab.



**Nuno Pereira:** Mechanical Engineer. Work experience in packaging and moulds. Working in Logoplaste since 2000. Coordinator of finite element department in Logoplaste Innovation Lab since 2007.



**Rudiney Souza:** Has a degree in Product Design. 14+ years experience in the plastics industry, namely in mold machining and product design, serves as CAD Manager in Logoplaste Innovation Lab since 2016.



**João Leitão:** MSc in Mechanical Engineering. Work experience in CFD and FEA consulting and production control. Working in packaging development for Logoplaste Innovation Lab since 2016.

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

2. ILAB works on research and development of high performance plastic packaging solutions in partnership with Arla, BP, Candia, CBPI, Coca Cola, Colgate-Palmolive, Danone, DM, Exxon Mobil, GSK, Heineken, Heinz, Henkel, Johnson&Johnson's, Lactalis, Lactogal, Nestlé, Nutrinveste, Olma, P&G, Reckitt Benckiser, SunnyD, Unicer, Unilever and Yoplait.
3. Poças, M.F.F.; Oliveira, J.C.; Pinto, H.J.; Zacarias, M.E.; Hogg, T. 2010. A novel approach for determining patterns of domestic usage of packaged food intake at home. British Food Journal Special Issue The New Food Choice and Consumer Paradigms, 112 (5), 500-510.
4. Poças, M.F.F.; Oliveira, J.C.; Pinto, H.J.; Zacarias, M.E.; Hogg, T. 2009. Characterization of Patterns of Food Packaging Usage in Portuguese Homes. Food Additives and Contaminants, 26 (9), 1314-1324.

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

4. Pollination Day. Open Innovation day organized by Logoplaste in Europe every 2 years where Customers, potential clients, Universities and Institutes are all invited to participate.



5. Biopolymer Seminar (tbd)
6. Packaging Seminar (tbd)

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e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

The following equipment is available for all Projects:

Design & CAD/CAE/CAM: Form Z RenderZone Plus, Varimetrix VX, Solidworks, Autodesk Inventor & Autocad

Finite Element Analysis: MSC Patran. Computer Aided Logistics: Cape Pack .Photorealistic 3D Rendering: 3D Studio Max Fast prototyping: Z Corporation Printer Z510, Dimension Elite 3D Printer.

The following industrial machines are available:

- Stretch blow molding – SIDEL SBO1 Lab1044; SIDEL SBO1-50; SIPA SFL 4/3 XL



- Extrusion blow molding – TECHNE Modul 2000; BEKUM BM 304SM



- Injection Molding – FERROMATIK KTEC 85S 2 cavity mold, standard and wide mouth preforms;



EURO M-544 — 3D contact and Optical System, A 200/15 — Vacuum Chamber for leak testing, C506-01-0002 — Top-Load and Volume check, ICC-2000 — Bust Test, PPT 3000 — Top-Load System Check, Vortex — Torque Measurement, OD 9500 — Vision Bottle Gauge (Dimensional Analyses), GAWIS OD 9500 — Vision Bottle Gauge (Dimensional Analyses), MM 8000 — Thickness measurement, C193-01-0001 — Thickness measurement, R 14 M — Shadow Graff, CMM Machine-Metris C3V 5.4.4 - 3D contact with Scanning contact probe, Mitutoyo PH-14A — Shadow Graph, ElektroPhysik Multitest 7200-FH4 — Wall thickness measurement, MultiTest 10-i — Top Load with wedge grippers for material characterization.

## INDUSTRY PARTNER PROFILE

### Section 1. General Partner Information

15. Partner name: MicroLife Solutions
16. Partner Website: [www.microlifesolutions.nl](http://www.microlifesolutions.nl)
17. Participant Identification Code (PIC) No: 924597502
18. Contact person name and email address: Tjalf de Boer – [Tjalf.deboer@microlifesolutions.nl](mailto:Tjalf.deboer@microlifesolutions.nl)
19. Position in organization: managing director
20. Department name: NA
21. Average Person Month Rate in the organization: 5 FTE



## INDUSTRY PARTNER PROFILE

### Section 2. Technical Part Contribution

a) Which is the role of your organization in the proposal? (*Explain in 2 sentences. Thank you.*)

MLS is involved in WP's 3, 4 and 5 (5 as WP lead) which deal with establishing microbial communities that degrade a mix of relevant plastics. We have access to a large collection of fungi and bacteria which will be tested for plastic degradability and used to build synthetic communities and MLS will be involved in supporting other partners with analyses such as DNA sequencing.

b) Which is the current state of the art of the technology you will introduce/progress during the project? *Please provide an overview of commercial solutions and initiatives in scientific community in the domain, patents and projects according to your knowledge*

White-rot fungi are saprotrophic fungi that break down recalcitrant plant polymers such as lignins and cellulose to consume as food. Especially lignin is a difficult molecule to break down as it has evolved as a defense against plant herbivores. These fungi achieve the breakdown of lignin by excreting extracellular enzymes such as laccase and mn-peroxidase that are notoriously substrate nonspecific. This non-specificity means that a whole range of compounds other than the natural substrates are also degraded by these fungi and their enzymes. We have found a white-rot fungus for example, that degrades 2,3,7,8-Tetrachlorodibenzodioxin which is one of the most recalcitrant dioxin congeners. Other studies have shown that white-rot fungal enzymes can degrade textile dyes, phenolic resins and plastics among other compounds and fungal laccase enzymes are commercially used in the pulping and textile industries. The role of MLS in the BioICEPT project is to study its current collection of some 80 white-rot fungal species for their ability to break down the different types of plastics and to integrate either the fungi or their enzymes into microbial consortia that are able to break down mixes waste streams of plastics.

c) Does your team have recent relevant scientific papers in this domain? (no more than 4 years old) Please provide a full list

1. Dao A.T.N., Vonck J., Janssens T.K.S., Dang H.T.C., Brouwer B. and de Boer T.E., (2019) Screening white-rot fungi for bioremediation potential of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *Industrial Crops & Products* 128(153-161).

2. Schulz-Bohm K., Tyc O., de Boer W., Peereboom N., Debets F., Zaagman N., Janssens T.K.S. and Garbeva P. (2017) Fungus-associated bacteriome in charge of their host behavior. *Fungal Genetics and Biology*. 102(38-48). Associated with document Ref. Ares(2019)6080743 - 01/10/2019

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

- BE-BASIC projects 7.3.6 and 7.3.7: MLS has been involved extensively in the national, public-private partnership funded research consortium BE-BASIC ([www.be-basic.org](http://www.be-basic.org)). Two specific projects, 7.3.6 and 7.3.7 are relevant for the BioICEPT project. In the 7.3.6 project white-rot fungi were investigated for their ability to degrade recalcitrant xenobiotics such as dioxins and in the 7.3.7 project fungi were used for lignin degradation in lignocellulose
- TKI-BIOCOM: A nationally funded project looking into enzymatic degradation of lignocellulosic biomass and recalcitrant fermentation inhibitors
- TKI-BIOCONSOLE: A nationally funded project investigating the use of cellulosomic enzyme complexes for simultaneously delignification and cellulose degradation.

e) Does your company provide related services or products as the ones mentioned in the project? *Please provide more information regarding the content and the clientele receiving this kind of services/products. Thank you.*

No

f) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

White-rot fungi and their extracellular enzymes are prime candidates for plastic polymer degradation but up till now degradation has only been demonstrated at lab scale and on single plastics. The challenges we face in this project are to get white-rot fungal species and enzymes to work together with other microorganisms to degrade a mix of different plastics.

j) Please provide info regarding the market of the technology that your organization will develop. Thank you. *(size of market, main competitors, costs of services/products etc.)*

MicroLife Solutions is looking to commercialise fungal strains and enzyme cocktails to degrade recalcitrant, anthropogenic chemicals in either the environment or in waste treatment processes. As there are many processes that need waste degradation (solid waste treatment such as plastics, wastewater treatment, manufacturing waste, etc) this market is potentially very large and depends on the range of compounds the fungal enzymes are able to degrade.

## INDUSTRY PARTNER PROFILE

### 3. Partner Profile Information

a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

MLS is a biotech start-up company with a mission to valorize valuable biological activities from nature's biochemical diversity such as bioactive small molecules and enzymes. The intended application markets range from bio-based industries, where biological catalysis can be implemented to make second-generation bio-based processes more sustainable, to the clean-up of xenobiotics in soil bioremediation, and aquaculture and plant production where novel natural alternatives are needed to combat pathogens and facilitate growth. By using methods such as HTP biological reporter assays, NGS and molecular biology tools, tailor-made solutions are provided for the discovery, small scale production, toxicological assessment and mechanistic unravelling of activities of interest and their respective formulations. MLS was raised in 2011 as a result of PPP's with academia in the EcoGenomics consortium. It falls under the BioDetection Systems (BDS) holding and it is physically embedded in the BDS infrastructure at the Amsterdam Science Park.

b) Relevant elements of the Curriculum Vitae/Biography (including profile pictures) of the team responsible for the project implementation (Please provide maximum two paragraphs per person and refer to the gender of each employee. Thank you.)

1. **Tjalf E. de Boer (Dr.)**, male, is managing and scientific director of MicroLife Solutions and is responsible for the general management, data analysis and bio-informatics. He has a background in molecular biology and bioinformatics and is steering the research activities within the PPP's towards the development of products and applications.
2. **Bram Brouwer (Prof. Dr.)**, male, is CEO of BDS and MLS, Managing Director and Board Member of the Dutch BE-Basic Foundation and he holds a chair in Environmental Toxicology and Ecogenomics at the VU University Amsterdam. Beside his expertise in toxicology, ecological genomics and assay development, he is renowned for his entrepreneurship by translating scientific knowledge to products for the global market. He is author (H-index 57) of more than 300 peer-reviewed articles in high ranking journals.
3. **Dao Ngoc Anh (MSc.)**, female, is a researcher on a joint scholarship between the Dutch BE-Basic consortium and the Vietnamese Academy of Science and Technology (VAST) and specialized in fungal biochemistry and biology.

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

3. Dao A.T.N., Vonck J., Janssens T.K.S., Dang H.T.C., Brouwer B. and de Boer T.E., (2019) Screening white-rot fungi for bioremediation potential of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *Industrial Crops & Products* 128(153-161).
4. Schulz-Bohm K., Tyc O., de Boer W., Peereboom N., Debets F., Zaagman N., Janssens T.K.S. and Garbeva P. (2017) Fungus-associated bacteriome in charge of their host behavior. *Fungal Genetics and Biology*. 102(38-48).
5. Janssens T. K. S., de Boer, T. E., Agamennone V., Zaagman N., van Straalen N.M., Roelofs D. (2017). Draft Genome Sequence of *Bacillus toyonensis* VU-DES13, Isolated from *Folsomia candida* (Collembola: Entomobryidae). *Genome announcements*. 5(19).
6. Ho A., Ijaz U.Z., Janssens T.K.S., Ruijs R., Kim S.Y., de Boer W., Termorshuizen A., van der Putten W.H., and Bodelier P.L.E. (2017) Effects of bio-based residue amendments on greenhouse gas emission from agricultural soil are stronger than effects of soil type with different microbial community composition. *Gcb Bioenergy*, 9(12), p1707-1720.

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

7. International Conference on Mycology, Fungi and Fungal Biology (ICMFFB)
8. BIO world congress (industrial biotechnology)

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

MicroLife solutions has standard lab facilities to culture fungi and bacteria in batches of up to 2 liters and the infrastucture to perform degradation experiments and screening of microbial consortia for plastic breakdown. We have access to HPLC and GC-MS-MS analysis to analyze breakdown products. MLS is planning to invest in a small bioreactor (up to 5 liters) for microbial culturing and protein purification equipment (FPLC).

## ACADEMIC PARTNER PROFILE

### Section 1. General Partner Information

1. Partner name: **National Technical University of Athens (NTUA)**
2. Partner Website: <https://www.chemeng.ntua.gr/indubiocat/>
3. Participant Identification Code (PIC) No: 999978142
4. Contact person name and email address: Evangelos Topakas, [vtopakas@chemeng.ntua.gr](mailto:vtopakas@chemeng.ntua.gr)
5. Position in organization: Assistant Professor
6. Department name: School of Chemical Engineering
7. Average Person Month Rate in the organization: 5700 euro

Logo of the  
organization





## Section 2. Technical Part Contribution

a) Which is the role of your organization in the proposal? (*Please explain in two sentences. Thank you.*)

NTUA will participate in three core WPs, such as WP 3, 4 and 5 and will also contribute to WP1 (Co-ordination and management) and WP8 (Dissemination and exploitation).

b) Which is the current state of the art of the technology you will introduce/progress during the project? Please provide a review of the recent scientific papers in the domain, patents and projects according to your knowledge. Thank you.

The group of NTUA will apply its expertise on assaying synthetic polymer degrading activities for allowing more efficient screening of microbial strains and enzymes. In addition, NTUA will work in the development of enzymatic processes for the breakdown/depolymerisation of mixed plastic waste materials. Novel enzyme discovery, protein isolation and heterologous expression are key features of the Dr Topakas group, which will be employed to enhance productivity of microbial depolymerisation of plastic polymers. A review of the recent literature has been already been given by Dr Nikodinovic.

c) Does your team have recent scientific papers in this domain? (no more than 4 years) (Please provide a full list. Thank you)

- Zerva A., Koutroufini E., Kostopoulou I., Detsi A., Topakas E. (2019). A novel thermophilic laccase-like multicopper oxidase from *Thermothelomyces thermophila* and its application in the oxidative cyclization of 2',3,4-trihydroxychalcone. *New Biotechnology*, 49, pp. 10-18.
- Karnaouri A., Antonopoulou I., Zerva A., Dimarogona M., Topakas E., Rova U., Christakopoulos P. (2019) Thermophilic enzyme systems for efficient conversion of lignocellulose to valuable products: structural insights and future perspectives for esterases and oxidative catalysts. *Bioresource Technology*, doi 10.1016/j.biortech.2019.01.062
- Kanelli M., Mandic M., Kalakona M., Vasilakos S., Kekos D., Nikodinovic-Runic J., Topakas E. (2018). Microbial production of violacein and process optimization for dyeing polyamide fabrics with acquired antimicrobial properties. *Frontiers in Microbiology*, 9:1495.
- Nikolaivits E., Dimarogona M., Karagiannaki I., Chalima A., Fishman A., Topakas E. (2018). Characterization and protein engineering of a novel versatile fungal polyphenol oxidase with chlorophenol bioremediation potential. *Applied and Environmental Microbiology*, 84(23), e01628-18.
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- Sunner H., Charavgi M-D., Olsson L., Topakas E., Christakopoulos P. (2015). Glucuronoyl esterase screening and characterization assays utilizing commercially available benzyl glucuronic acid ester. *Molecules*, 20, pp. 17807-17817.
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- Kanelli M., Douka A., Vouyiouka S., Papaspyrides C.D., Topakas E., Papaspyridi L.-M., Christakopoulos P. (2014). Production of biodegradable polyesters via enzymatic polymerization and solid-state finishing. *Journal of Applied Polymer Science*, 131(19), pp 1-8.

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

- NoWasteBioTech (2018-2020): Novel Conversion Technologies of Waste Biomass to Food additives and Fine Chemicals, funded by Hellenic Foundation for Research & Innovation. The project involves the use of enzymes and microorganisms for the production of high added value compounds from waste byproducts
- Volatile (2016-2020): Biowaste derived volatile fatty acid platform for biopolymers, bioactive compounds and chemical building blocks, funded by the European Commission under the Horizon 2020 program (Call H2020-NMBP-BIO-2016 Grant agreement No. 720777). NTUA leads the use of fermentation technology for the production of fatty acids from waste byproducts
- IKY-DAAD (2018-2019): "Enzymatic superficial modification of natural and synthetic polymers and their spectroscopic analysis" funded by German Academic Exchange Service. NTUA works on the development of novel enzymes with oxidative activity and their role in functionalization and surface modification of polymers.
- TasteSTEVIA (2018-2020): Holistic approach along the production cycle of *Stevia rebaudiana* plant cultivated in Greece, via combined application of innovative methods of precision agriculture and bitter aftertaste removal techniques, funded by EPANAK 2014-2020 Operational Programme, Competitiveness-Entrepreneurship-Innovation. NTUA is involved in the use of biocatalysis for the enzymatic bioconversion of stevia sugars for improving the aftertaste of the final product.
- TASCAR (2015-2019): Tools And Strategies to access original bioactive compounds by Cultivating MARine invertebrates and associated symbionts, funded by Horizon 2020 research and innovation programme, under grant agreement No 634674. NTUA works on the discovery of novel enzymes for biocatalysis from marine fungi.
- OPTIBIOCAT (2013-2017) Optimized esterase biocatalysts for cost-effective industrial production, KBBE.2013.3.3-04 FP7 Collaborative project. NTUA works on the use of biocatalysis for the enzymatic bioconversion of bioactive compounds.
- TEXTENZ (2011-2013): Upgrading of Textile products using novel enzyme activities, SYNERGASIA 2009, funded by the Greek Ministry of Economy, Competitiveness and Shipping. NTUA is involved in the use of biocatalysis for the enzymatic modification of synthetic polymers.

Development and discovery of the novel biocatalytic system (enzymatic and/or microbial) for the degradation of a variety of plastic polymers and mixes of thereof. Our long experience on cutinases and generally on members of the superfamily of serine esterases, as well as oxidative enzymes, will aid in the design of a target strategy for the efficient plastics bioremediation.

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

NTUA will contribute in WPs 3, 4, and 5 with a wide range of activities, as reported to the following tasks:

- T3.1 Production and chromatographic purification of selected enzymes on 20 -50 mg scale for the screening experiments
- T3.3 Establishment of novel assays for screening microbial enzymes for plastics degradation potential (plate and liquid assays using model compounds and their defined mixtures) and surface degradation analysis
- T3.4 Purification and biochemical characterization of novel enzymatic activities
- T3.5 Immobilization of target enzymatic activities (Biocatalyst stabilization)
- T3.7 Construction of a microbial platform for 'microbial-cell factory' using Systems Biocatalysis approach
- T4.1 Biodiscovery screen of (existing and new) microbial biobanks
- T4.2 Isolation of new microbes and biobank enrichment
- T4.3 Construction of a single microbial platform for boosting plastics degradation capacity
- T4.4. Liquid media cultivation with standard plastics
- T4.5. Liquid media cultivation with pre-treated plastics
- T4.6. Quantitative/qualitative analysis of plastic breakdown potential and dynamics



- T4.8. Material Transfer Agreement
- T5.1 Establishment of the stable mixed community of bacteria and fungi suitable for biological pre-treatment of mixed plastic waste coming from WP2.
- T5.2 Forming synthetic communities using established plastic degrading strains
- T5.3 Forming and enrichment of relevant, natural communities to increase their plastic degradation potential
- T5.5 Identification of plastic transformation and breakdown products from degradation by microbial consortia

h) Which are the deliverables that your organization will deliver during the project?

- D3.1 Report on the screening of pure enzymatic activities for their potential to degrade plastics
- D3.3 Demonstrate of new accelerated screening by novel in situ biosensors which flag high performing
- D3.4 Establishment of novel assays for screening plastics degradation activities
- D3.5 Report on mechanism of enzymatic and/or microbial attack on the pre-treated plastics
- D3.6 Discovery of at least two novel enzymatic activities capable of degrading plastics
- D3.7 Report on the enzyme or whole cell immobilization of target biocatalysts
- D3.9 Construction of the microbial cell factory for the conversion of plastics waste into valuable products
- D4.1 Isolation and establishment of new biobank microbial strains
- D4.2. Identification of microbial degraders of plastics from existing and new biobanks
- D4.3. Generation of novel strains with boosted plastic degradation capacities
- D4.4 Establishment of liquid cultivation conditions for pre-identified degraders on standard and pre-treated plastics
- D4.5 Characterisation of depolymerase enzyme activities after liquid cultivation
- D4.6. Quantitative and qualitative characterisation of plastic breakdown potential and dynamics
- D4.8 Consolidated identification and recommendation of the best microbes and their growth conditions to support WP4, WP5 and WP6 for various up-scaling optimisations.
- D4.9 Sharing of identified strains, their growth conditions for consortia optimisation after signing of appropriate Material Transfer Agreement (MTA)
- D5.1. Information on plastic degradation enrichment properties and potential of existing microbial communities.
- D5.2. Information on synthetic community vs individual microbe performance for plastic breakdown based on combined data from WP3 and WP5.
- D5.3. Information on minimal, optimized community composition and plastic degrading enzymes present in these communities based on (meta)genomic DNA sequencing.
- D5.4. Optimized synthetic and enriched natural plastic degradation microbial communities which will breakdown at least 20% of relevant, non-biodegradable plastics.

i) Which are the technical objectives that your organization needs to achieve during the project?

- Discovery of novel enzymatic activities through enzyme isolation and characterization/protein identification
- Production of pure enzymes and screening for the ability to depolymerize a variety of plastic polymers
- Defining and testing tailor-made cocktails of enzymes that can be used at various stages of pre-treatment and after pre-treatment of mixed plastic waste
- Improvement of enzymes/biocatalysts properties using engineering approaches (directed evolution, immobilisation, etc.)
- Evaluation and assessment of enzymatic depolymerisation products

j) Please provide info regarding the market of the technology that your organization will develop (*size of market, main competitors, costs of services/products etc.*)

There is no market for the biocatalytic degradation of plastics. The present EU proposal aims in a high risk and relatively low TRL project that probably, if successful, will create a new market on plastics bioremediation.

## ACADEMIC PARTNER PROFILE

### 3. Partner Profile Information

a) Description of the organization and its services

Associated with document Ref. Ares(2019)6080743 - 01/10/2019

*(The organization description should be in accordance with the undertaken tasks in the project)*

**National Technical University of Athens (NTUA)** is the top Technical University in Greece. Today NTUA has more than 7000 students, employs 700 persons as academic staff and more than 2500 researchers. Based on 2010 Euro Research Ranking Data, NTUA reached 10th place between educational organizations and 3rd position on networking rank (reputation). NTUA is coordinating or participating in several European projects and received 400 million EUR funding from European Commission in the last decade. **Biotechnology Laboratory** is a part of School of Chemical Engineering (<https://www.chemeng.ntua.gr/en>) that has a renowned world reputation in the area of biomass bioconversion and biochemistry of plant cell wall degrading enzymes for the production of bioactive compounds and biofuels.

b) Relevant elements of the Curriculum Vitae/Biography (*including profile pictures*) of the team responsible for the project implementation. *(Please provide maximum 2 paragraphs per person and refer to the gender of each employee. Thank you)*

**1) Dr. Evangelos Topakas – M (40% of full time planned)**



0000-0003-0078-5904

PhD in Industrial Biotechnology, National Technical University of Athens (2004)

Present position: Assistant Professor, School of Chemical Engineering, NTUA

Research experience: **Discovery of novel enzymes** (cellulolytic, hemicellulolytic and ligninolytic enzymes) for the enzyme-aided extraction or modification of bioactive components (Biocatalysis in non-conventional media) from biomass using conventional and modern bioinformatics assisted strategies (genome mining of *Fusarium oxysporum* and *Sporotrichum thermophile*), heterologous overexpression (*Pichia pastoris*, *Escherichia coli*) and **biochemical characterization** of carbohydrate degrading recombinant enzymes, **study of the structure/function relationship and regulatory mechanisms of the enzymes induced by saprophytic organisms**. Emphasis is given on the utilization of residual biomass for the production of 2nd generation liquid biofuels and high-added value compounds.

Publications: 101

**2) Dr. Anastasia Zerva – F (40% of full time planned)**



0000-0003-0361-7690

PhD in Biotechnology, National Technical University of Athens (2017)

Present position: Postdoctoral Researcher, School of Chemical Engineering, NTUA

Research experience: **Development of novel biocatalyst preparations and biocatalytic processes** for the production of value-added compounds from renewable lignocellulosic sources. **Heterologous expression and characterization** of novel oxidative enzymes from lignocellulolytic microorganisms, focusing on their valorization as biocatalysts for the synthesis of novel bioactive compounds and/or polymers.

Publications: 12

### 3) Dr. Anthi Karnaouri – F (40% of full time planned)



0000-0001-9164-7667

PhD in Biotechnology, National Technical University of Athens (2015)

Present position: Postdoctoral Researcher, School of Chemical Engineering, NTUA

Research experience: **Characterization of novel biocatalysts** implicated on bioconversion of carbohydrates. Cloning, expression and production of enzymes implied in the degradation/modification of carbohydrates from biomass, biochemical studies and enzyme characterization, enzymatic modification/depolymerization of biomass fractions. **Development of enzymatic and fermentation technologies** for the valorization of different types of biomass feedstocks and fractions recovered in different streams after pretreatment processes.

Publications: 16

### 4) Dr. Stamatina Vouyiouka – F (40% of full time planned)



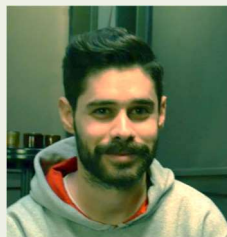
PhD in Polymer Technology, National Technical University of Athens (2004)

Present position: Assistant Professor, School of Chemical Engineering, NTUA

Research experience: **Polymerization processes and recycling**, with a special emphasis on polyamides/polyesters including bio-based and/or biodegradable materials. **Combination of enzymatic pre-polymerization with bulk post-polymerization** towards properties upgrade and development of sustainable production methods. **Development of encapsulation processes** of active ingredients in polymer nano- and/or microparticles to induce functionalities in polymeric materials.

Publications: 37

### 5) Efstratios Nikolaivits – M (40% of full time planned)



0000-0002-8022-9272

BSc in Chemical Engineering, National Technical University of Athens (2014)

Present position: PhD candidate in Biotechnology, School of Chemical Engineering, NTUA

Research experience: **Discovery and characterization of novel biocatalysts** with bioremediation potential.

Publications: 9

**6) Constantine Papaspyrides – M (20% of full time planned)**



0000-0002-3901-0041

PhD in Polymer Science and Technology, National Technical University of Athens (1982)

Present position: Professor, School of Chemical Engineering, NTUA

Research experience: **Polyamides / polyesters science and technology**. Green solid state polycondensation processes.; Migration of additives from plastics in food. Packaging / "Nano"-Packaging. ; Flame Retardancy. **Polymers and Nanotechnology**. Nanocatalysis. Nanocomposites. Flame Retardancy; Polymers and Biotechnology. Biodegradable Polymers; **Waste management and recycling of plastics including food packaging**. Recycling and Migration. Recycling of polymeric composite materials; **Polymeric composite Materials / Flexible Textile Structures**: Preparation and End-Properties Tailoring.

Publications: 149

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

1. Zerva A., Koutroufina E., Kostopoulou I., Detsi A., Topakas E. (2019). A novel thermophilic laccase-like multicopper oxidase from *Thermothelomyces thermophila* and its application in the oxidative cyclization of 2',3,4-trihydroxychalcone. *New Biotechnology*, 49, pp. 10-18.
2. Nikolaivits E., Dimarogona M., Karagiannaki I., Chalima A., Fishman A., Topakas E. (2018). Characterization and protein engineering of a novel versatile fungal polyphenol oxidase with chlorophenol bioremediation potential. *Applied and Environmental Microbiology*, 84(23), e01628-18.
3. Nikolaivits E., Norra G-F, Voutsas E., Topakas E. (2016). Cutinase from *Fusarium oxysporum* catalyzes the acylation of tyrosol in an aqueous medium: optimization and thermodynamic study of the reaction. *Journal of Molecular Catalysis B, Enzymatic*, 129, pp.29-36.
4. Dimarogona M., Nikolaivits E., Kanelli M., Christakopoulos P., Sandgren M., Topakas E. (2015). Structural and functional studies of a *Fusarium oxysporum* cutinase with polyethylene terephthalate modification potential. *Biochimica et biophysica Acta*, 1850, 2308-2317.
5. Kanelli M., Douka A., Vouyiouka S., Papaspyrides C.D., Topakas E., Papaspyridi L.-M., Christakopoulos P. (2014). Production of biodegradable polyesters via enzymatic polymerization and solid-state finishing. *Journal of Applied Polymer Science*, 131(19), pp 1-8.

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you

6. 15th International Symposium on Biocatalysis and Biotransformations (BioTrans 2021)
7. ICEBB 2020: International Conference on Environmental Biotechnology and Bioremediation

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

Biotechnology Laboratory (BL) covers a surface area of 400 m<sup>2</sup> and possesses the following equipment, which is necessary for the project implementation: PCR, electrophoresis units, 2D-PAGE system suitable for the analysis and investigation of secretoms, incubators, well equipped enzyme reactors and bioreactors (solid state and submerged batch) ranging from 1-120 L (10 bioreactors), protein purification system (FPLC), analytical instrumentation (HPLC, GC, HPAEC, FTIR), Ultrafiltration Minitan system. Apart from BL equipment, facilities from the Horizontal Laboratory of the School of Chemical Engineering are available, such as GC-MS, LC-MS, NMR. BL has a close collaboration and access to the facilities of the Polymer Laboratory that include equipment for study of polymerization processes, polymer modification and material characterization, such as molecular weight determination, thermal analysis, mechanical and rheological properties.

Equipment for production of recombinant enzymes



Equipment for characterization of polymer properties (TGA, GC-MS, rheological properties)



## ACADEMIC PARTNER PROFILE

### Section 1. General Partner Information

1. Partner name: The Provost, Fellows, Foundation Scholars, and the other members of Board, of the College of the Holy and Undivided Trinity of Queen Elizabeth near Dublin (TCD), hereinafter "Trinity College Dublin (TCD)"
2. Partner Website: [www.tcd.ie](http://www.tcd.ie) ; <http://ambercentre.ie/>
3. Participant Identification Code (PIC) No: 999845446
4. Contact person name and email address (Technical): Dr. Ramesh Babu, [babup@tcd.ie](mailto:babup@tcd.ie)
5. Position in organization: Principal Investigator
6. Department name: School of Physics and AMBER
7. Average Person Month Rate in the organization: 5169



**Trinity College Dublin**  
Coláiste na Tríonóide, Baile Átha Cliath  
The University of Dublin

## ACADEMIC PARTNER PROFILE



a) Which is the role of your organization in the proposal? *(Please explain in two sentences. Thank you.)*

TCD will be leading the WP2 along with AIT, CUT, AMPLAS and AVCOM in developing and optimizing the various pre-treatment processes for individual and mixed plastic waste to enhance the microbial degradation and valorization

b) Which is the current state of the art of the technology you will introduce/progress during the project? Please provide a review of the recent scientific papers in the domain, patents and projects according to your knowledge. Thank you.

This task aims to advance biological treatment of plastic waste beyond the state of the art by developing the circular pre-treatment processes to provide substrates suitable for microbial degradation and also by designing value added polymer blends, to extract a valorisable carbon source. Currently, plastic waste is pre-treated by thermal (REF), Chemical (REF), Photo Catalytic (REF) and Mechanical (REF) pre-treatment processes to enhance the microbial degradation. However, currently, none of the current pre-treatment processes add value during the pre-treatment process. In order to maximize the valorisation of plastic waste, residual degraded products arising from pre-treatment processes will be captured by extracting them into ecofriendly green solvents. Also solid pre-treated plastic waste arising from pre-treatment (WP2) and after microbial degradation (WP3) will be evaluated as compatibilisers to create high value polymer blends suitable for 3D printing applications.

c) Does your team have recent scientific papers in this domain? (no more than 4 years) (Please provide a full list. Thank you)

- Production of bacterial nanocellulose (BNC) and its application as a solid support in transition metal catalysed cross-coupling reactions (2019); doi.org/10.1016/j.ijbiomac.2019.01.154.
- Biodegradable plastic blends create new possibilities for end-of-life management of plastic but they are not a panacea for plastic pollution (2018); Environmental science and Technology; 52 (18) 10441-10452
- Filtering Media by Electrospinning: Next generation Membranes for separation applications (2018) by Springer; Chapter 8: Affinity membranes for capture of cells and biological substances; ISBN: 978-3-319-78163-1
- Surfactant-mediated hydrothermal pretreatment of Ryegrass followed by enzymatic saccharification for polyhydroxyalkanoate production (2018); Industrial Crops and Products (111): 625-632
- Use of a mannitol rich ensiled grass press juice (EGPJ) as a sole carbon source for polyhydroxyalkanoates (PHAs) production through high cell density cultivation (2015), Bioresource Technology 191: 45-52.
- Pervaporation separation of butyric acid from aqueous and anaerobic digestion (AD) solutions using PEBA based composite membranes (2015), J. Industrial and Engineering Chemistry, 23:163-170
- High cell density cultivation of Pseudomonas putida KT2440 using glucose without the need for oxygen enriched air supply (2015), Biotechnology and Bioengineering 112: 725-733.

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

Dr. Ramesh is previously involved in various EU grants including DESYGNIT (FP6), SYNPOL (FP7), AgriChemWhey (BBI-JU flagship) grants. Currently he is actively involved in number of national centres: the Advanced Materials and BioEngineering Research Centre (AMBER), the Bioeconomy Research Centre (BEACON), Food for health Ireland (FHI), and Dairy Processing Technology (DPTC) Centre for developing sustainable materials and processes across different sectors. He is also a founding member of the Irish Bioeconomy Foundation (IBF) which is a vehicle to promote the Bio- and circular economy in Ireland. Dr. Ramesh's work in AMBER includes collaboration with Glanbia, Medtronic, Mergon, Innovative Polymer Compounds, Millipore, Rogers Corporation, and Western Digital, developing novel polymer composites for medical devices, membranes, separation processes, bio based composites, barrier materials and composites for automotive applications.

e) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

i) Enabling the accessibility of plastic waste to microbial communities for biodegradation to tackle global plastic waste problem

ii) Identifying the methods to recover useful carbon sources (monomers, oligomers and other degraded products) from pre-treatment, enzymatic and microbial degradation processes for valorization



g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- Design various pre-treatment methods to modify the physical and chemical properties of the non-degradable plastic waste to attach the microorganisms to the surface of the plastic waste.
- Improve the hydrophilicity, incorporation of functional groups, introduction of unsaturation for mixed plastic waste using various pre-treatment methods.
- Monitoring the physical, chemical, thermal and mechanical properties of the mixed plastic waste to monitor the efficiency of pre-treatment process.
- Designing the downstream processes for the recovery of carbon source from various pre-treatment processes.
- Formulating novel polymer blends suitable for 3D printing
- Improve the pre-treatment technology process from TRL3 to TRL5 for selected plastic waste mix to generate highest yield (X%) of carbon feed stock to produce upscaled products (PHA, bacterial cellulose, other materials).
- .....
- Task 1: Designing and Characterization of plastic waste feedstock composition:
- Plastic waste vary in their composition depend on their geographical location from source to source
- .....
- Task2: pre-treatment processes and optimization
- Task 3:Characterisation of pre-treated plastic waste
- Task4: Extraction and purification
- Task5: Production of tailored and high performance polymer blends for 3D printing
- .....

h) Which are the deliverables that your organization will deliver during the project?

D1.Mixed plastic waste Feed stock characterization report and supply plan of pre-treated samples to partners [M12]

- D2. New pre-treatment process for plastic waste report[ M36]
- D3. Report on the composition of recovered carbon from various pre-treatment methods [M28].
- D4. Technical report on properties of circular polymers created and their suitability for 3D printing [M36]
- D5. Pre-treatment process economics data report.
- .....

i) Which are the technical objectives that your organization needs to achieve during the project?

1. Develop the pre-treatment methods to enhance the biodegradation of plastic waste.
2. Develop the methods for separation and recovery of carbon compounds from pre-treatment processes.
3. Characterization of plastic waste before and after pre-treatment processes
4. Formulating and evaluating polymer blends for 3D printing using the pre-treated plastic waste as a compatibiliser with selected polymers

...

j) Please provide info regarding the market of the technology that your organization will develop *(size of market, main competitors, costs of services/products etc. )*

.....Not Applicable

## ACADEMIC PARTNER PROFILE

### 3. Partner Profile Information

a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

Founded in 1592, Trinity College Dublin (TCD) is recognized internationally as Ireland's leading university, ranked 104<sup>th</sup> in the World, and 29<sup>th</sup> in Europe, in the 2018/2019 QS World University Ranking. In addition, Trinity is ranked in the top 15% internationally by QS for citations (2017), and is a member of the prestigious League of European Research Universities (LERU). Trinity's research strategy is based on the identification and promotion of multi-disciplinary research areas in which the College has a critical mass of world-class researchers, which have potential to make significant scientific and economic impact. The College currently has 24 Schools across 3 faculties: Arts, Humanities and Social Sciences; Engineering, Mathematics and Science; and Health Sciences. Its current flagship interdisciplinary research institutes are in areas such as neuroscience, nanoscience and materials science, biomedical science, international integration studies, and arts and humanities. The construction of Ireland's first purpose-built nanoscience research institute (CRANN) was opened in January 2008 and it hosts the Advanced Materials and BioEngineering Research Centre (AMBER), which comprises a team of over 350 researchers across 7 institutions, led by 41 Principal Investigators, each of whom is an internationally recognized expert in their field of research. The AMBER Centre is funded by Science Foundation Ireland, with co-funding from 38 industry partners. These include Merck, Johnson & Johnson, Pepsico and Intel.

b) Relevant elements of the Curriculum Vitae/Biography (*including profile pictures*) of the team responsible for the project implementation. (*Please provide maximum 2 paragraphs per person and refer to the gender of each employee. Thank you*)

Dr. Saranya Ramesh Kumar (Female)

Prof. Mick Morris (Male)

Dr. Ramesh Babu (Male)

**Dr. Saranya Rameshkumar (Female)** is presently a research fellow in the CRANN institute, School of Physics, Trinity College Dublin (TCD). She received her doctorate in the year 2016 from National Institute of Technology-Tiruchirappalli, India for her PhD dissertation on the topic "Performance evaluation of nanomaterials incorporated mixed matrix membranes" with the main focus on improving membrane performance for recovering value-added components from industrial effluents. After PhD, she extended her research as a post-doctoral researcher in Indian Institute of Chemical Technology, Hyderabad, India with a start-up research grant funded by Science and Engineering Research Board (SERB), India. She has gained 6 years of research experience on developing polymeric nanocomposite membranes; bench-scale membrane process for separation and water/wastewater treatment applications. She has published 10 research papers in the international peer-reviewed journals, 4 book chapters and also presented her research in International Conferences.

**Dr Ramesh Babu (Male)** leads the Polymeric Material Nanocomposites Group (PMNC) at Trinity College Dublin. The group is primarily engaged in applied research, providing the R&D capability to allow companies to become involved in the use of the most advanced polymeric materials and tools to create smart products and technology to compete effectively in all markets. Graduated from the University of Mumbai, India in 1998 with a Ph.D. in Chemistry. Currently, he leads Polymer materials and Nanocomposite group in School of Physics, CRANN and AMBER centre. He has over 17 years of experience in polymer processing, membrane separation, nanocomposites, biodegradable polymers and polymer characterisation. He has worked in Clariant GmbH and Asahi-Kasei Corporation, Japan before joining the Materials Ireland Polymer research Centre, Trinity College Dublin, in 2003. He has published over 40 international journal papers, conference papers, 10 patent families and 4 patents. He is at the forefront of Industrial research representing TCD in various technology centers funded national funding bodies and working with various industry partners across the globe. Dr. Ramesh is a funded investigator in AMBER, the national centre of excellence in materials science, BEACON Bioeconomy research Centre, Dairy Processing Technology Centre (DPTC) and Food for health Ireland.

**Prof. M A Morris (Male)** is the Director of the AMBER Research Centre, Ireland's Materials Research Centre. AMBER is a \$100 million centre covering research areas of nanoelectronics, biomaterials, polymer science, sensors and characterisation. M A Morris leads the nanoelectronics theme. AMBER work with several international companies including Intel, Nokia Bell laboratories, Analog and Johnson and Johnson. AMBER has dedicated world class facilities for nanofabrication, electron microscopy and additive manufacture. **Prof. Morris has published >300 papers with citations >10,000 and an H-index of 55.** Prof. Morris contributed to the 2013 and 2015 ITRS as a member of the Emerging Materials Panel. His work has made a major

contribution to the field of block copolymer self-assembly and lithography as witnessed by grants from the Semiconductor Research Council (SRC) and the first European scientists to have three or more grant awards. Prof Morris has been funded continuously by Intel for over 15 years and developed technologies with Intel including ultra-low dielectric constant materials. Prof. Morris has been awarded personal grants of ~€25 million as well as centre funding of €80 million.

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

Kataria, R. and Woods, T. and Casey, W. and Cerrone, F. and Davis, R. and O'Connor, K. and Ruhal, R. and Babu, R., Surfactant-mediated hydrothermal pretreatment of Ryegrass followed by enzymatic saccharification for polyhydroxyalkanoate production, *Industrial Crops and Products*, 111, 2018, p625-632 Journal Article, 2018 [DOI](#)

Heinrich, D. and Raberg, M. and Fricke, P. and Kenny, S.T. and Morales-Gamez, L. and Babu, R.P. and O'Connor, K.E. and SteinbÄEchel, A., Synthesis gas (syngas)-derived medium-chainlength polyhydroxyalkanoate synthesis in engineered *Rhodospirillum rubrum*, *Applied and Environmental Microbiology*, 82, (20), 2016, p6132-6140 Journal Article, 2016 [DOI](#)

Reddy, C.S. and Oâ Connor, K. and Babu P, R., The Influence of Biobased Olegomeric Diisocyanate on Thermal and Mechanical Properties of Poly(3-hydroxybutyrate), *Macromolecular Symposia*, 365, (1), 2016, p223-229 Journal Article, 2016 [DOI](#)

Walsh, M. and O'Connor, K. and Babu, R. and Woods, T. and Kenny, S., Plant oils and products of their hydrolysis as substrates for polyhydroxyalkanoate synthesis, *Chemical and Biochemical Engineering Quarterly*, 29, (2), 2015, p123-133 Journal Article, 2015 [DOI](#)

Davis, R. and Duane, G. and Kenny, S.T. and Cerrone, F. and Guzik, M.W. and Babu, R.P. and Casey, E. and O'Connor, K.E., High cell density cultivation of *Pseudomonas putida* KT2440 using glucose without the need for oxygen enriched air supply, *Biotechnology and Bioengineering*, 112, (4), 2015, p725-733 Journal Article, 2015 [DOI](#)

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

1. World convention on recycling and reuse, USA
2. Materials Research Society conference on plastics
3. Plastic Europe
4. RAPID+TCT –Accelerating 3D manufacturing
5. EU Circular Plastics Alliance – we are closely following developments in this recent EU initiative

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

The PMNC laboratory has an excellent suite of infrastructure to carry out research in various aspects of polymeric membranes, polymer processing and characterization (<https://www.tcd.ie/Physics/pmnc/facilities/>). Particularly for this project, PMNC is equipped with lab scale UV exposure set up, Brabender melt mixer (50g), ScCo2 chamber and continuous 1500W ultrasonicator system to perform various pre-treatment processes. The group has dedicated membrane lab consists different filtration units (5L-100L) based on different types of polymeric and ceramic membranes which can be used to recover the carbon from pre-treatment processes. Also the group is equipped with Brabender KTSE 20/40 twin extruder with 5-10kg/hr processing capacity with pelletiser.



Other characterization facilities includes, such as TGA, DSC, DMA, GPC, FTIR, contact angle, NANON Electrospinner, Volume/Surface Resistivity (8009 Kiethley Test Fixture), Mechanical Analysis (Zwick Roell - Instron), Oxygen Permeation Analyser (Systech Illinois - OTR), Melt Flow Index MFI (Karg Industrietechnik), Thermal Conductivity (Armfield HT10XC Transfer Unit), Viscometer (Brookfield DVI-Prime), and Shore Hardness tester. Together with the key assets given above, the group also have full access 3D printing laboratory and to the multi-million euro microscopy facility, the Advanced Microscopy Lab (SEM, TEM facility). <http://www.crann.tcd.ie/Facilities/Advanced-Microscopy-Laboratory.aspx> located in Trinity college Dublin.

## ACADEMIC PARTNER PROFILE

### Section 1. General Partner Information

1. Partner name: **Beijing Institute of Technology**
2. Partner Website: <http://www.bit.edu.cn/index.htm>
3. Participant Identification Code (PIC) No: **10007**
4. Contact person name and email address: **Dr. Yu Yang**; [yooyoung@bit.edu.cn](mailto:yooyoung@bit.edu.cn)
5. Position in organization: **Professor**
6. Department name: **Department of Biology, School of Life Science**
7. Average Person Month Rate in the organization: **10**



Associated with document Ref. A/2019/13 - 01/10/2019



## ACADEMIC PARTNER PROFILE

### Section 2. Technical Part Contribution

a) Which is the role of your organization in the proposal? *(Please explain in two sentences. Thank you.)*

We are contributing as WP leader and research provider for WP1 on the “Isolation of synthetic plastic-degrading microorganisms and identification of key depolymerases” in China part.

b) Which is the current state of the art of the technology you will introduce/progress during the project? Please provide a review of the recent scientific papers in the domain, patents and projects according to your knowledge. Thank you.

During this project, we will use the plastic as sole carbon source to enrich the plastic-degrading microbial community, and then analyze the microbial composition by using metagenomics sequencing. With the taxonomic information of the species in the enrichment, we could isolate the microorganisms in the pure culture. Then we could characterize the plastic-degrading capability of these isolates.

c) Does your team have recent scientific papers in this domain? (no more than 4 years) (Please provide a full list. Thank you)

- Peng Ruiting, Xia Mengli, Ru Jiakang, Huo Yixin, **Yang Yu\***. Microbial degradation of polyurethane plastics. *Chin J Biotech*, 2018, 34(9): 1398–1409.
- **Yang Yu**, Yang Jun, Jiang Lei. Comment on “A bacterium that degrades and assimilates poly(ethylene terephthalate)”. *Science*, 2016, 353(6301): 759.
- **Yang Yu**, Yang Jun, Wu Wei-Min, Zhao Jiao, Song Yiling, Gao Longcheng, Yang Ruifu, Jiang Lei. Biodegradation and mineralization of polystyrene by plastic-eating mealworms. 1. Convinced evidence. *Environmental Science & Technology*, 2015, 49(20): 12080-12086.
- **Yang Yu**, Yang Jun, Wu Wei-Min, Zhao Jiao, Song Yiling, Yang Ruifu, Jiang Lei. Biodegradation and mineralization of polystyrene by plastic-eating mealworms. 2. Role of gut microorganism. *Environmental Science & Technology*, 2015, 49(20): 12087-12093.
- **Yang Yu**, Chen Jianwei, Wu Wei-Min, Zhao Jiao, Yang Jun. Complete genome sequence of *Bacillus* sp. YP1, a polyethylene-degrading bacterium from waxworm’s gut. *Journal of Biotechnology*, 2015, 200: 77–78.

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

- National Nature Science Foundation of China (51603004)
- Young Elite Scientist Sponsorship Program of the China Association of Science and Technology (No. 2017QNRC001)
- National Key Research and Development Program of China (2016YFC1402504)

e) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

Plastic pollution has become a global environmental issue, making it necessary to explore the environmental disposal technology for plastic waste. During this project, we will try to isolate the novel plastic-degrading fungi and bacteria, and identify the key depolymerases and the corresponding degradation products. These findings will contribute to the development of high efficient biological disposal for plastic waste.

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*



Associated with document Ref. Ares(2019)6080743 - 01/10/2019

1. Isolation of new plastic-degrading strains from environmental samples.
2. Identification of novel depolymerases and the corresponding coding genes from the plastic-degrading strains.
3. Determination of the high resolution crystal structure of the novel depolymerase and the relationship between the structure and function.

h) Which are the deliverables that your organization will deliver during the project?

1. Plastic-degrading strains
2. Depolymerases
3. Crystal structure data of the depolymerases

i) Which are the technical objectives that your organization needs to achieve during the project?

1. The method for the identification of depolymerase
2. The method for the crystallization of depolymerase

j) Please provide info regarding the market of the technology that your organization will develop *(size of market, main competitors, costs of services/products etc. )*

## ACADEMIC PARTNER PROFILE

### 3. Partner Profile Information

a) Description of the organization and its services

*(The organization description should be in accordance with the undertaken tasks in the project)*

In 1940, Beijing Institute of Technology (BIT), the first science and engineering university was founded in Yan'an by the Communist Party of China. It has been one of the key universities in China since the founding of New China and the first batch of universities which has entered the national "211 Project", "985 Project" and the "Top A World-class University". The name of BIT was inscribed by Chairman Mao, and Li Fuchun, Xu Teli, Li Qiang and other older generations of proletarian revolutionists successively took the lead of the university. In the 2018 World University Rankings published by QS (Quacquarelli Symonds), BIT ranked 389th in the world, 76th in Asia and 17th in mainland China. The university is now affiliated to the Ministry of Industry and Information Technology. All the faculty members and students are striving for the goal of "two hundred years" of the national standard, and are fully committed to the goal of building a world-class university with Chinese characteristics.

b) Relevant elements of the Curriculum Vitae/Biography *(including profile pictures)* of the team responsible for the project implementation. *(Please provide maximum 2 paragraphs per person and refer to the gender of each employee. Thank you)*



Dr. Yu Yang is currently working as a professor at Department of Biology, School of Life Science, Beijing Institute of Technology.



In 2009, he began the research on the topic of “Biodegradation of plastic by the plastic-eating insects and their gut microorganisms”. He first demonstrated that the plastic-eating waxworm and mealworms could degrade polyethylene and polystyrene. After then, he found that the gut symbiont of these plastic-eating insect play a key role in the plastic digestion in the gut. Up to now, he has isolated 3 plastic-degrading bacteria from the plastic-eating insect. These findings pave a new way for isolating more other novel plastic-degrading microorganisms from the insect gut. He has published 20 peer reviewed paper in the journal such as *Science*, *Nature Communications* and *Environmental Science & Technology* so on. The citations are more than 300 times. 2017, He was award as the excellent young scholar by the Chinese Society for Environmental Chemistry and for the Best Research Paper by the Chinese Society for Environmental Microbiology. He is currently principal investigator for three projects funded by National Nature Science Foundation of China (NSFC), China Association of Science and Technology (CAST) and Ministry of Science and Technology of China (MOST).

Dr. Yixin Huo is currently working as a professor at Department of Biology, School of Life Science, Beijing Institute of Technology.



Supported by a French Government CNOUS Fellowship, in 2005, Dr. Huo received Ph.D. degrees simultaneously from both University of Paris 7 and Peking University before joining Prof. James C. Liao's laboratory at UCLA as a postdoctoral research fellow. At UCLA, he was the first to demonstrate a carbon and nitrogen neutral biofuel production process, a milestone that was a featured cover story in *Nature Biotechnology* and highlighted by *Nature Chemical Biology* and *Nature*. Since joining Easel Biotechnologies, LLC in 2011, he has served as a group leader for the ARPA-E funded Electrofuel project in collaboration with UCLA. Dr. Huo was selected as a recipient by “The Recruitment Program of Global Youth Experts” in 2014, and became a full professor in Beijing Institute of Technology since Oct 2015. Dr. Huo has long term experience with the microbial refinery and production of natural resources by using multiple model microorganisms. The researches focused on the “design-construction-screening-scale up” of the microbial cell factories, and the applicant had 1) demonstrated the first successful example of converting the carbon skeleton of protein biomass into value-added chemicals. The nitrogen-related metabolic networks in the host strains were re-built by creating multiple irreversible amino acid deamination pathways; 2) developed a genetically engineered *Ralstonia eutropha* strain to store electrical energy as chemical energy in biofuels or biochemical; 3) clarified the mechanisms of the protein-protein interactions between RNA polymerase and special regulatory factors during the biological nitrogen fixation and stress response; 4) been awarded a U.S. DOE (Department of Energy) project, and successfully converted the cellulosic hydrolysates into biofuels and value-added chemicals using engineered microorganisms through synthetic biology strategies. He had published over 20 papers as first author or leading senior author in high profile international scientific journals such as *Science*, *Nature Biotechnology*, *Nature Communications*, and *Nucleic Acids Research*. He had multi-year experiences in industry and successfully commercialized several technologies. His patents in biofuel production are the basis for Easel's commercialization processes and have been tested for the industrial production. He has given approximately 30 presentations at several of the most prestigious international conferences.

c) Please provide a list of up to 5 relevant publications, products and/or services. Thank you.

1. Peng Ruiting, Xia Mengli, Ru Jiakang, Huo Yixin, **Yang Yu\***. Microbial degradation of polyurethane plastics. *Chin J Biotech*, 2018, 34(9): 1398–1409.
2. **Yang Yu**, Yang Jun, Jiang Lei. Comment on “A bacterium that degrades and assimilates poly(ethylene terephthalate)”. *Science*, 2016, 353(6301): 759.
3. **Yang Yu**, Yang Jun, Wu Wei-Min, Zhao Jiao, Song Yiling, Gao Longcheng, Yang Ruifu, Jiang Lei. Biodegradation and mineralization of polystyrene by plastic-eating mealworms. 1. Convinced evidence. *Environmental Science & Technology*, 2015, 49(20): 12080-12086.
4. **Yang Yu**, Yang Jun, Wu Wei-Min, Zhao Jiao, Song Yiling, Yang Ruifu, Jiang Lei. Biodegradation and mineralization of polystyrene by plastic-eating mealworms. 2. Role of gut microorganism. *Environmental Science & Technology*, 2015, 49(20): 12087-12093.
5. **Yang Yu**, Chen Jianwei, Wu Wei-Min, Zhao Jiao, Yang Jun. Complete genome sequence of *Bacillus* sp. YP1, a polyethylene-degrading bacterium from waxworm's gut. *Journal of Biotechnology*, 2015, 200: 77–78.

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

1. AAAS 2020 Annual Meeting

Associated with document Ref. Ares(2019)6080743 - 01/10/2019

2. ASM Microbe 2019

3. 15th International Symposium on Biocatalysis and Biotransformations (BioTrans 2021)

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

1. LC/MS-MS
2. GC/MS-MS (EI & CI)
3. GCFID/TCD
4.  $H^1$ -NMR and  $C^{13}$ -NMR
5. HPLC -Rapid Resolution
6. Freeze Dryer
7. PCR
8. Protein Purification System
9. Gel Electrophoresis System (including Gel Doc Unit)
10. Solid Phase Extraction Manifold
11. SEM
12. Environmental Growth Chambers (2x Walk-In + 2x Reach-In)

## ACADEMIC PARTNER PROFILE

### Section 1. General Partner Information

8. Partner name: Shandong University
9. Partner Website: <http://www.en.sdu.edu.cn/>
10. Participant Identification Code (PIC) No: 10422
11. Contact person name and email address: qiqingsheng@sdu.edu.cn
12. Position in organization: Professor
13. Department name: State Key laboratory of Microbial Technology
14. Average Person Month Rate in the organization:



## ACADEMIC PARTNER PROFILE

### Section 2. Technical Part Contribution

- a) Which is the role of your organization in the proposal? *(Please explain in two sentences. Thank you.)*

SDU will participate the WP4, WP3, WP5 and WP6 to develop enzymatic and biocatalytic solutions for single and mixed plastic degradation; establish a catalogue of high performance microbial strains for plastic degradation and bioplastic production; establish high performance microbial consortia for plastic degradation and bioplastic production; and bioprocess value-added biopolymer products

b) Which is the current state of the art of the technology you will introduce/progress during the project? Please provide a review of the recent scientific papers in the domain, patents and projects according to your knowledge. Thank you.

...We will construct the metabolic pathways from the degraded monomers to bulk chemicals, and will set up the microbial communities to convert plastic to biopolymer

c) Does your team have recent scientific papers in this domain? (no more than 4 years) (Please provide a full list. Thank you)

Qian Wang, Jiasheng Xu, Zhijie Sun, Yaqi Luan, Ying Li, Junshu Wang, Quanfeng Liang, Qingsheng Qi: *Engineering an in vivo EP-bifido pathway in Escherichia coli for high-yield acetyl-CoA generation with low CO<sub>2</sub> emission*. Metabolic Engineering 08/2018; 51., DOI:10.1016/j.ymben.2018.08.003

Zhiyong Cui, Zhennan Jiang, Jinhong Zhang, Huihui Zheng, Xin Jiang, Kai Gong, Quanfeng Liang, Qian Wang, Qingsheng Qi: *Stable and efficient biosynthesis of 5-aminolevulinic acid using plasmid-free Escherichia coli*. Journal of Agricultural and Food Chemistry 01/2019;, DOI:10.1021/acs.jafc.8b06496

Zhiyong Cui, Xin Jiang, Huihui Zheng, Qingsheng Qi, Jin Hou: *Homology-independent genome integration enables rapid library construction for enzyme expression and pathway optimization in Yarrowia lipolytica*: CUI et al.. Biotechnology and Bioengineering 11/2018;, DOI:10.1002/bit.26863

Zedao Liu, Jizhong Zhang, Jiao Jin, Zilong Geng, Qingsheng Qi, Quanfeng Liang: *Programming Bacteria With Light—Sensors and Applications in Synthetic Biology*. Frontiers in Microbiology 11/2018; 9:2692., DOI:10.3389/fmicb.2018.02692

Tianyuan Su, Haiying Jin, Yi Zheng, Qian Zhao, Yizhao Chang, Qian Wang, Qingsheng Qi: *Improved ssDNA recombineering for rapid and efficient pathway engineering in Corynebacterium glutamicum*. Journal of Chemical Technology & Biotechnology 06/2018;, DOI:10.1002/jctb.5726

Xue Zhang, Jian Zhang, Jiasheng Xu, Qian Zhao, Qian Wang, Qingsheng Qi: *Engineering Escherichia coli for efficient coproduction of polyhydroxyalkanoates and 5-aminolevulinic acid*. Journal of Industrial Microbiology and Biotechnology 12/2017; 45(8), DOI:10.1007/s10295-017-1990-4

Pengfei Gu, Xiangyu Fan, Quanfeng Liang, Qingsheng Qi, Qiang Li: *Novel technologies combined with traditional metabolic engineering strategies facilitate the construction of shikimate-producing Escherichia coli*. Microbial Cell Factories 12/2017; 16(1), DOI:10.1186/s12934-017-0773-y

Peng Yang, Jing Wang, Qingxiao Pang, Fengyu Zhang, Junshu Wang, Qian Wang, Qingsheng Qi: *Pathway optimization and key enzyme evolution of N-acetylneuraminic acid biosynthesis using an in vivo aptazyme-based biosensor*. Metabolic Engineering 08/2017; 43., DOI:10.1016/j.ymben.2017.08.001

Jian Pang, Zhan-Ying Liu, Min Hao, Yong-Feng Zhang, Qing-Sheng Qi: *An isolated cellulolytic Escherichia coli from bovine rumen produces ethanol and hydrogen from corn straw*. Biotechnology for Biofuels 06/2017; 10(1), DOI:10.1186/s13068-017-0852-7

Zhiyong Cui, Cuijuan Gao, Jiaojiao Li, Jin Hou, Carol Sze Ki Lin, Qingsheng Qi: *Engineering of unconventional yeast Yarrowia lipolytica for efficient succinic acid production from glycerol at low pH*. Metabolic Engineering 06/2017; 42., DOI:10.1016/j.ymben.2017.06.007

Jiaojiao Li, Yikui Li, Zhiyong Cui, Quanfeng Liang, Qingsheng Qi: *Enhancement of succinate yield by manipulating NADH/NAD(+) ratio and ATP generation*. Applied Microbiology and Biotechnology 01/2017; 101(8), DOI:10.1007/s00253-017-8127-6

Xinyuan He, Yan Chen, Quanfeng Liang, Qingsheng Qi: *An autoinduced AND-gate controlling metabolic pathway dynamically in response to microbial communities and cell physiological state*. ACS Synthetic Biology 12/2016; 6(3), DOI:10.1021/acssynbio.6b00177

Tianyuan Su, Fapeng Liu, Pengfei Gu, Haiying Jin, Yizhao Chang, Qian Wang, Quanfeng Liang, Qingsheng Qi: *A CRISPR-Cas9 Assisted Non-Homologous End-Joining Strategy for One-step Engineering of Bacterial Genome*. Scientific Reports 11/2016; 6:37895., DOI:10.1038/srep37895

Yizhao Chang, Tianyuan Su, Qingsheng Qi, Quanfeng Liang: *Easy regulation of metabolic flux in Escherichia coli using an endogenous type I-E CRISPR-Cas system*. Microbial Cell Factories 11/2016; 15(1), DOI:10.1186/s12934-016-0594-4

Cuijuan Gao, Xiaofeng Yang, Huaimin Wang, Cristina Perez Rivero, Chong Li, Zhiyong Cui, Qingsheng Qi, Carol Sze Ki Lin: *Robust succinic acid production from crude glycerol using engineered Yarrowia lipolytica*. Biotechnology for Biofuels 08/2016; 9(1):179., DOI:10.1186/s13068-016-0597-8

Xiaoli Yu, Haiying Jin, Xuelian Cheng, Qian Wang, Qingsheng Qi: *Transcriptomic analysis for elucidating the physiological effects of 5-aminolevulinic acid accumulation on Corynebacterium glutamicum*. Microbiological Research 08/2016; 192., DOI:10.1016/j.micres.2016.08.004

Pengfei Gu, Tianyuan Su, Qian Wang, Quanfeng Liang, Qingsheng Qi: *Tunable switch mediated shikimate biosynthesis in an engineered non-auxotrophic Escherichia coli*. Scientific Reports 07/2016; 6:29745., DOI:10.1038/srep29745

Fengyu Zhang, Jiaojiao Li, Huaiwei Liu, Quanfeng Liang, Qingsheng Qi: *ATP-Based Ratio Regulation of Glucose and Xylose Improved Succinate Production*. PLoS ONE 06/2016; 11(6):e0157775., DOI:10.1371/journal.pone.0157775

Dongfang Gao, Yaqi Luan, Quanfeng Liang, Qingsheng Qi: *Exploring the N-terminal role of a heterologous protein in secreting out of Escherichia coli: A non-classical signal peptide in protein secretion*. Biotechnology and Bioengineering 06/2016; 113(12)., DOI:10.1002/bit.26028

Peng Yang, Wenjing Liu, Xuelian Cheng, Jing Wang, Qian Wang, Qingsheng Qi: *A new strategy for the production of 5-aminolevulinic acid in recombinant Corynebacterium glutamicum with high yield*. Applied and Environmental Microbiology 02/2016; 82(9)., DOI:10.1128/AEM.00224-16

Pengfei Gu, Tianyuan Su, Qingsheng Qi: *Novel technologies provide more engineering strategies for amino acid-producing microorganisms*. Applied Microbiology and Biotechnology 01/2016; 100(5)., DOI:10.1007/s00253-015-7276-8

• .....

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

• no.....

e) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

.....efficiently setting up the microbial communities to convert plastic to biopolymer

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- Screening/selection of suitable host for production of PHA/SA/nanocellulose
- Engineering/evolution of the host for utilization of various substrates (monomers from different plastics)
- Engineering of the host for the production of bulk chemicals/polymers
- Setting up the microbial communities to degrade the plastic efficiently
- Setting up the microbial communities to convert the plastic to biopolymers (PHA/SA/etc)
- Establishment of the stable mixed community of bacteria and fungi suitable for biological treatment of mixed plastic waste
- Forming synthetic communities using established plastic degrading strains
- Forming and enrichment of relevant natural communities
- Taxonomic identification of microbial consortia members and sequencing of metagenomes
- Establishment of defined microbial consortia for the simultaneous plastic degradation and product
- Protocol for optimal breakdown parameters.
- Bioprocesses optimization in laboratory scale
- Process validation at 10L reactor laboratory scale
- Construction of pilot reactor according to specification input from WP2, WP3 and WP5.
- Pilot reactor set up
- Pilot reactor operation
- Monitoring or pilot reactor operation and production process
- 

h) Which are the deliverables that your organization will deliver during the project?

- Report on plastic degradation enrichment properties and potential of existing microbial communities.

- Report on synthetic community vs individual microbe performance for plastic breakdown based on combined data from WP3 and WP5.
- Report on minimal, optimized community composition and plastic degrading enzymes present in these communities based on metagenomic DNA sequencing.
- Establishment of optimized synthetic and enriched natural plastic degradation microbial communities which will breakdown at least 20% of relevant, non-biodegradable plastics.
- Information on plastic breakdown products to be fed as carbon sources into the fermentation processes developed by WP6.
- Report on the best strain and process operation conditions to produce the target products.
- Report on the metabolic model and monitoring for process optimization
- Samples and protocols for the production of PHBs, nanocellulose and rhamnolipids with high performance mechanical and chemical properties suitable for processing for high value end use product.
- Operation of modular integrated BioICEP pilot scale plant demonstrating the biocatalytic and microbial breakdown of 20%+ of mixed plastics.
- Small scale pilot production of high performance PHB and nanocellulose for applications such as food packaging and rhamnolipids for pharmaceutical applications.

i) Which are the technical objectives that your organization needs to achieve during the project?

- To develop enzymatic and biocatalytic solutions for single and mixed plastic degradation
- To establish a catalogue of high performance microbial strains for plastic degradation and bioplastic production.
- Formation of stable microbial communities suitable for surface modifications of recalcitrant plastics.
- Formation of new enriched selected communities with increased plastic degradation capacities.
- Establishment of the microbial community platform with coupled degradation-synthetic capabilities
- Monitoring of the microbial community composition and performance during various stages of bioprocesses.
- Development of bioprocesses for the production of PHB with distinct monomer composition and functional properties, using waste synthetic plastics' monomers as feedstock
- Development of bioprocesses for the production of nanocellulose using waste synthetic plastics' monomers as feedstock.
- Development of bioprocesses for the production of different types of rhamnolipids, using waste synthetic plastics' constituent molecules and monomers as feedstock.
- Process optimization by online monitoring and metabolic modelling
- Protocols for the preparation of bioproduct with properties with high performance characteristics for application in end use products as achieved using a feedstock process with chemical, mechanical, and thermal analysis and WP3, WP4 and WP5.
- Establishment of integrated automatized small scale BioICEP pilot plant
- Operation of the BioICEP pilot plant for 20%+ mixed plastics degradation
- Small scale pilot production of high performance PHB and nanocellulose for applications such as food packaging and rhamnolipids for pharmaceutical applications.

j) Please provide info regarding the market of the technology that your organization will develop (*size of market, main competitors, costs of services/products etc.* )

N/A.....

### 3. Partner Profile Information



*(The organization description should be in accordance with the undertaken tasks in the project)*

...The State Key Laboratory for Microbial Technology (SKLMT) was formally established at Shandong University in November 1995 with the financial aids of World Bank and the Chinese Government.

The main research interest of the Laboratory is microbial technology related to the sustainable development of human society, especially on the engineering of micro-organisms with the diverse physiological functions using gene engineering, protein engineering, metabolic engineering, etc.

#### **Biomass Resource Conversion**

☐ conversion of lignocellulose and organic solvents or wastes to useful products employing the related micro-organisms; discovering the reaction mechanisms during these processes.

#### **Resource & Environmental Microbiology**

☐ screening the new micro-organisms from the environment, including Myxobacteria, marine bacteria, bacteria under extreme environment, etc; discovering the evolution relationship and interaction mechanisms in ecosystem;

☐ exploiting the novel application of these microorganisms.

#### **Molecular and Synthetic Microbiology**

☐ modification of micro-organisms by genetic engineering, metabolic engineering and synthetic biology towards the production of useful products, such as biopolymers, functional foods, etc.

b) Relevant elements of the Curriculum Vitae/Biography (*including profile pictures*) of the team responsible for the project implementation. *(Please provide maximum 2 paragraphs per person and refer to the gender of each employee. Thank you)*



Dr. Qingsheng Qi, received his Ph.D in University of Muenster, Germany. Then he became a staff member in University of Chemnitz, Germany. Since 2004, he is full professor in State Key Laboratory of Microbial Technology in Shandong University, People's Republic of China. Meanwhile, he is joint professor of National Glycoengineering Research Center.

Dr. Qi has made many important contributions in setting up metabolic pathways of polyhydroxyalkanoates in *E. coli*. His current research interest is focusing on metabolic engineering and synthetic biology of microorganisms. Specifically, these interest includes: 1) Pathway engineering of micro-organisms towards the efficient production of useful bulk chemicals, including PHA, and value added compounds; 2) Screening the new microbes and new enzymes with specific properties; 3) Developing the metabolic and biosynthetic methods or tools for the synthetic biology.

c) Please provide a list of up to 5 relevant publications, products and/or services.

1. Qian Wang, Jiasheng Xu, Zhijie Sun, Yaqi Luan, Ying Li, Junshu Wang, Quanfeng Liang, Qingsheng Qi: *Engineering an in vivo EP-bifido pathway in Escherichia coli for high-yield acetyl-CoA generation with low CO<sub>2</sub> emission*. Metabolic Engineering 08/2018; 51., DOI:10.1016/j.ymben.2018.08.003.....



2. Zhiyong Cui, Xin Jiang, Huihui Zheng, Qingsheng Qi, Jin Hou: *Homology-independent genome integration enables rapid library construction for enzyme expression and pathway optimization in Yarrowia lipolytica*: CUI et al.. Biotechnology and Bioengineering 11/2018;, DOI:10.1002/bit.26863
3. Peng Yang, Jing Wang, Qingxiao Pang, Fengyu Zhang, Junshu Wang, Qian Wang, Qingsheng Qi: *Pathway optimization and key enzyme evolution of N -acetylneuraminate biosynthesis using an in vivo aptazyme-based biosensor*. Metabolic Engineering 08/2017; 43., DOI:10.1016/j.ymben.2017.08.001
4. Zhiyong Cui, Cuijuan Gao, Jiaojiao Li, Jin Hou, Carol Sze Ki Lin, Qingsheng Qi: *Engineering of unconventional yeast Yarrowia lipolytica for efficient succinic acid production from glycerol at low pH*. Metabolic Engineering 06/2017; 42., DOI:10.1016/j.ymben.2017.06.007
5. Quanfeng Liang, Qingsheng Qi: *From a co-production design to an integrated single-cell biorefinery*. Biotechnology Advances 11/2014; 32(7)., DOI:10.1016/j.biotechadv.2014.08.004.....

d) Please list 2-3 relevant conferences/events that you would like to target for presenting your results. Thank you.

1. 15th International Symposium on Biocatalysis and Biotransformations (BioTrans 2021)
2. Symposium on Biotechnology for Fuels and Chemicals 2021.

e) Please describe any significant infrastructure and/or equipment to be used in the project. Thank you.

- PCR
- Incubator
- Centrifuge
- Spectrophotometer
- HPLC
- Aminex HPX-87H ion exclusion column
- Gas chromatograph

## ACADEMIC PARTNER PROFILE

### Section 1. General Partner Information

1. Partner name: Institute of Microbiology, Chinese Academy of Sciences
2. Partner Website: [www.im.ac.cn](http://www.im.ac.cn)
3. Participant Identification Code (PIC) No: 12100000400012318X
4. Contact person name and email address:  
Prof. Shuang-Yan Tang    [tangsy@im.ac.cn](mailto:tangsy@im.ac.cn)  
  
Prof. Yong Tao            [taoyong@im.ac.cn](mailto:taoyong@im.ac.cn)
5. Position in organization: Professor
6. Department name: CAS Key Laboratory of Microbial Physiological and Metabolic Engineering
7. Average Person Month Rate in the organization: 15,000 RMB



## ACADEMIC PARTNER PROFILE

a) Which is the role of your organization in the proposal?

We are responsible for the engineering of plastics degradation enzymes and construction of the recombinant strain for efficiently degrading mixed plastics.....

b) Which is the current state of the art of the technology you will introduce/progress during the project? Please provide a review of the recent scientific papers in the domain, patents and projects according to your knowledge. Thank you.

We will engineer the enzymes through both rational design and directed evolution. We will develop biosensors of the degradation products of different kind of synthetic plastics, and use them as high-throughput screening tools for the directed evolution of the degrading enzymes.....

c) Does your team have recent scientific papers in this domain? (no more than 4 years) (Please provide a full list. Thank you)

- .....1. Wei Chen<sup>#</sup>, Xuanxuan Zhang<sup>#</sup>, Dandan Xiong<sup>#</sup>, Jian-Ming Jin<sup>\*</sup>, **Shuang-Yan Tang<sup>\*</sup>**. Engineering the effector specificity of regulatory proteins for the in vitro detection of biomarkers and pesticide residues. *Applied Microbiology and Biotechnology* 2019, doi: 10.1007/s00253-019-09679-1.
2. Heng Li, Jing Li, Ruinan Jin, Wei Chen, Chaoning Liang, Jieyuan Wu, Jian-Ming Jin<sup>\*</sup>, **Shuang-Yan Tang<sup>\*</sup>**, Towards the construction of high-quality mutagenesis libraries. *Biotechnology Letters* 2018, **40**: 1101-1107.
3. Heng Li, Wei Chen, Ruinan Jin, Jian-Ming Jin<sup>\*</sup>, **Shuang-Yan Tang<sup>\*</sup>**, Biosensor-aided high-throughput screening of hyper-producing cells for malonyl-CoA-derived products. *Microbial Cell Factories* 2017, **16**: 187.
4. Heng Li, Chaoning Liang, Wei Chen, Jian-Ming Jin<sup>\*</sup>, **Shuang-Yan Tang<sup>\*</sup>**, Yong Tao, Monitoring *in vivo* metabolic flux with a designed whole-cell metabolite biosensor of shikimic acid. *Biosensor and Bioelectronics* 2017, **98**: 457-465.
5. Jieyuan Wu, Peixia Jiang, Wei Chen, Dandan Xiong, Linglan Huang, Junying Jia, Yuanyuan Chen, Jian-Ming Jin<sup>\*</sup>, **Shuang-Yan Tang<sup>\*</sup>**, Design and application of a lactulose biosensor. *Scientific Reports* 2017, **7**: 45994.
6. Qingzhuo Wang, **Shuang-Yan Tang<sup>\*</sup>**, Sheng Yang<sup>\*</sup>. Genetic biosensors for small-molecule products: Design and applications in high-throughput screening. *Frontiers of Chemical Science and Engineering* 2017, **11**: 15-26.
7. Dandan Xiong, Shikun Lu, Jieyuan Wu, Chaoning Liang, Wei Wang, Wenzhao Wang, Jian-Ming Jin<sup>\*</sup>, **Shuang-Yan Tang<sup>\*</sup>**, Improving key enzyme activity in phenylpropanoid pathway with a designed biosensor. *Metabolic Engineering* 2017, **40**: 115-123.
8. Wei Chen<sup>#</sup>, Shan Zhang<sup>#</sup>, Peixia Jiang<sup>#</sup>, Jun Yao, Yongzhi He, Lincai Chen, Xiwu Gui, Zhiyang Dong<sup>\*</sup>, **Shuang-Yan Tang<sup>\*</sup>**. Design of an ectoine-responsive AraC mutant and its application in metabolic engineering of ectoine biosynthesis. *Metabolic Engineering* 2015, **30**:149-155.

• .....

d) Have you been involved in the last decade to a National or European Project related with the technology you develop in this project or in the same domain? *If so, please mention the funding scheme, the acronym and a brief overview of what you developed in this project. Thank you.*

• No.....

e) Which are the scientific and technical obstacles that your organization will try to resolve during the project? *Please refrain from using generalities (i.e. global problem of hunger)*

.....Development of super high-throughput screening methods for the plastics degrading enzymes engineering.....

g) Which are the tasks you will undertake in the project? *(Please provide a full description as these parts will be added in the Work Packages)*

- Establishment of the stable mixed community of bacteria and fungi suitable for biological treatment of mixed plastic waste
- Forming synthetic communities using established plastic degrading strains.
- .....

h) Which are the deliverables that your organization will deliver during the project?

- Plastics degrading enzyme mutants with improved activities and recombinant strains expressing all the highly-active plastics degrading enzymes.....

- Report on plastic degradation enrichment properties and potential of existing microbial communities
- Report on synthetic community vs individual microbe performance for plastic breakdown based on combined data from WP3 and WP5.
- Report on minimal, optimized community composition and plastic degrading enzymes present in these communities based on metagenomic DNA sequencing.
- Establishment of optimized synthetic and enriched natural plastic degradation microbial communities which will breakdown at least 20% of relevant, non-biodegradable plastics.
- Information on plastic breakdown products to be fed as carbon sources into the fermentation processes developed by WP6.
- .....

i) Which are the technical objectives that your organization needs to achieve during the project?

- Development of high-throughput screening methods for plastics degrading enzymes engineering and obtaining of enzyme mutants with improved activities
- Formation of stable microbial communities suitable for surface modifications of recalcitrant plastics.
- Development of new enriched selected communities with increased plastic degradation capacities.
- .....

j) Please provide info regarding the market of the technology that your organization will develop (*size of market, main competitors, costs of services/products etc.* )

N/A.....

## ACADEMIC PARTNER PROFILE

### 3. Partner Profile Information

#### ACTECO (ACT)

Acteco is an environmental company with dedicated experience in collecting and treating all kind of waste, plastic, food waste, oil, cardboard, (350.000 ton/year) hazardous waste (44.00 ton/year), contaminate water. We produce 12.000 ton/year of recycling plastic. We have developed systematic recycling technologies and our polymer sorting and processing technology will play a key role in this project. A combination of mixed plastic wastes will be designed to promote the optimization of microbial degradation. We will also assist in providing polymer wastes.

We provide comprehensive environmental consulting and advice services

- We recycle and pellet plastics, including ABS, polypropylene, polyethylene, polystyrene.
- We also collect and transport waste from our customers
- We also supply waste optimisation equipment, such as container, compacters, bailers, industrial parts cleaning machines, rotocompactors and waste cages.

Biography of the team responsible for the project implementation

Angel Martinez Leon - Aeronautical Engineer Research in more than 10 different R&D project

Luis Gonzalez – Agricultural Engineer Research in 4 different R&D project

Francisco Colomina. Chemical Degree Reserch in 4 different R&D project

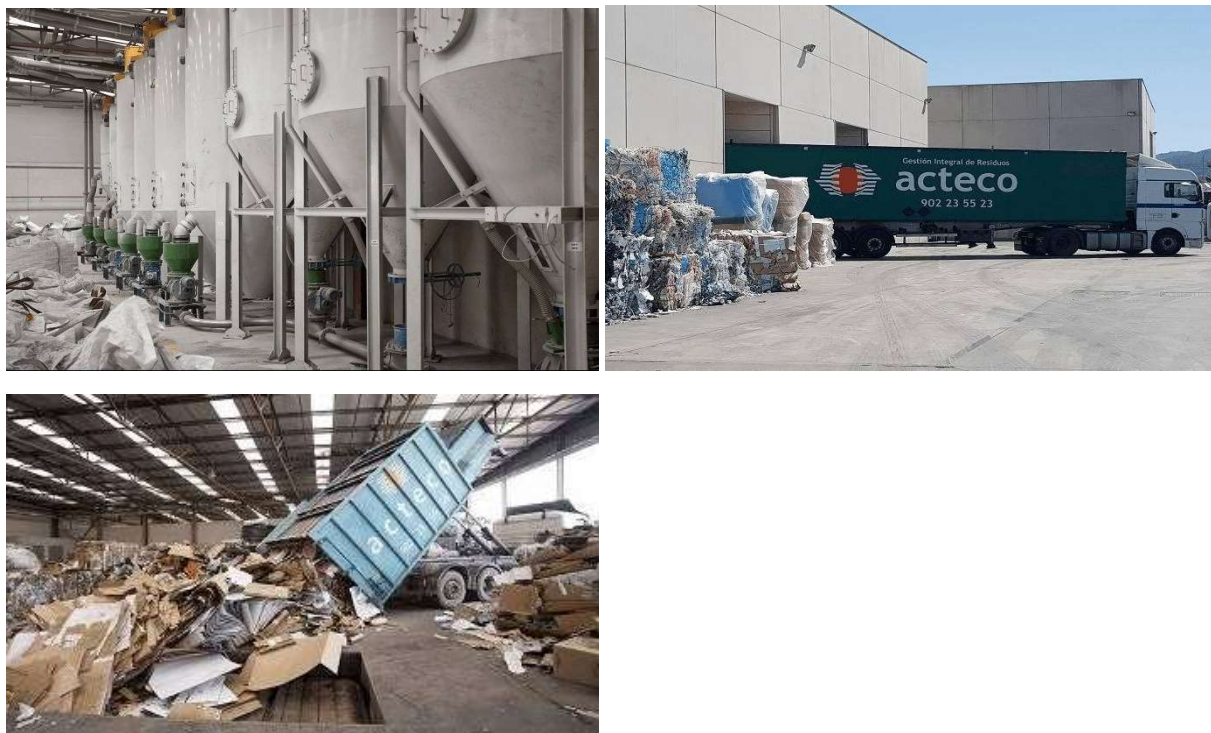
Nuria Llopis. Chemical Degree Reserch in 3 R&D project. Expertise in Food contact industry

3 relevant conferences/events that you would like to target for presenting your results. Thank you.

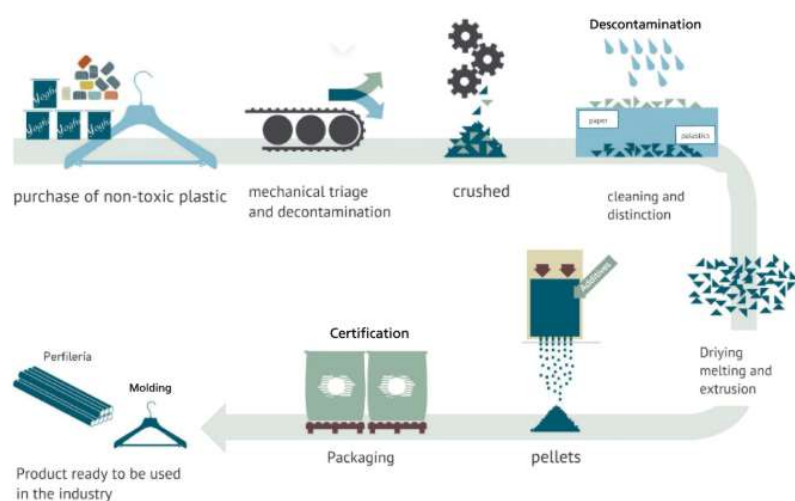
9. Empresas que cambian el mundo Congreso de los Diputados Madrid 2018

10. Suchem Congres Universidad de Zaragoza 2017.

significant infrastructure and/or equipment to be used in the project. Thank you.



Our recycling process



#### 4.2. Third parties involved the project (including use of third party resources)

*This topic is part of the EU-China flagship initiative on Biotechnology for Environment and Human Health, which will promote substantial coordinated and balanced research and innovation cooperation between the EU and China.*

The International partners are all RPO's and comprise three Chinese Universities, SDU, BIT and CAS. Each of these international partners play an instrumental role in the research planned and is clearly detailed in the WP tasks. The contributions by the three Chinese partners is financed by a 15 million-yuan RMB budget from the National Natural Sciences Foundation of China (NSFC). This is equivalent to 1.94 million Euro.

In table 40 4.2 a brief description of the international partners, the tasks they will perform and an estimation of the costs involved is provided. International partners will be linked to the coordinator and in 870292 BioICEP - Part B

certain WP will work with different EU partner in order to ensure responsibility for their compliance with their respective obligations.

Table 39: 4.2 Third party contributions, tasks to be performed and estimated costs

No.	International Partners	Abbrev.	Nation	Status	Estimated Costs
13	Beijing Institute of Technology Beijing Institute of Technology is one of the key universities in China since the founding of New China and the first batch of universities which has entered the national "211 Project", "985 Project" and the "Top A World-class University".	BIT	China	RPO	€635,641
14	Shandong University The State Key Laboratory for Microbial Technology (SKLMT) was formally established at Shandong University in November 1995 and is dedicated to the development of microbial technology related to the sustainable development of human society.	SDU	China	RPO	€635,641
15	Institute Of Microbiology Chinese Academy of Sciences The Institute of Microbiology of the Chinese Academy of Sciences (IMCAS) is the largest microbiological research institution in China. It was founded on December 3, 1958, through the merger of the Institute of Applied Mycology and the Beijing Laboratories of Microbiology, both of which were affiliated to the Chinese Academy of Sciences (CAS)	CAS	China	RPO	€635,641

**Coordinating Institution: No. 1 Athlone Institute of technology (AIT)**

Question(s):	Reponse:
Do any of the participants plan to subcontract certain tasks? <i>(please note that core tasks of the project should not be sub-contracted)</i> <i>If yes, please describe and justify the tasks to be subcontracted</i>	No
Do any of the participants envisage that part of its work is performed by linked third parties? <i>If yes, please describe the third party, the link of the participant to the third party, and describe and justify the foreseen tasks to be performed by the third party</i>	No
Do any of the participants envisage the use of contributions in kind provided by third parties? (Articles 11 and 12 of the General Model Grant Agreement) <i>If yes, please describe the third party and their contributions</i>	No
Do any of the participants envisage that part of the work is performed by International Partners <sup>29</sup> (Article 14a of the General Model Grant Agreement)? <i>If yes, please describe the International Partner(s) and their contributions</i>	Yes
<p>The international partners are three Chinese institutes .BIT, SDU or CAS. The international partners are linked to the coordinator,AIT, and in certain WP will work with different EU partners in order to ensure responsibility for their compliance with their respective obligations</p> <p>BIT is linked to the coordinator AIT and will carry out work in each of the WP 1, and WP 3-8 as described in detail in the WP descriptions.</p> <p>SDU is linked to the coordinator AIT and will carry out work in each of the WP 1, and WP 3-8 as described in detail in the WP descriptions. This work primarily involves screening microorganisms to obtain highly efficient plastic degradation strains and the produce high-value biopolymers and bioproducts from degradation waste plastic carbon sources</p> <p>CAS is linked to the coordinator AIT and will carry out work in each of the WP 1, WP 3-6 and WP 8 as described in detail in the WP descriptions. This work primarily involves identifying genes encoding degradation enzymes with high activities,</p>	

END OF SECTION 4



## 5. Ethics and Security

**5.1 Ethics.** An Ethics package for the project will be prepared and made available to all of consortium partners allowing them to fulfil the ethics requirements procedures for the BioICEP project.

In order to comply with the ethics requirements, maintained documentation of required risk analysis, ethics approvals and authorisations, licencing and legal obligations will be carried out. The processes outlined are in compliance with EU policy. The documentation is from the jurisdiction of Ireland and is in compliance with the relevant EU policies. Each EU member state has equivalent documents and procedures in place which may also be used for these purposes.

### Ethics Requirements

- An ethics advisor will be appointed which will liaise with the AIT ethics committee. This advisor will maintain an overview of the work throughout the whole course of the BioICEP project and will assist in checking for compliance with relevant ethical standards, facilitating the probity of the BioICEP research activities and reporting to the co-ordinator and to the Commission. A documented opinion from the AIT Ethics committee or other appropriate ethics structure in an EU consortium country confirming that the research activity can be legally carried out in an EU country will be provided and kept on file.
- For Serbia and China as non-EU countries within the BioICEP consortium a risk-benefit analysis will be provided on their research activities, which involves micro-organism sample collection from plastic waste sites and investigation and promotion of these microbes for waste plastic biodegradation and fermentation. Copies of ethics approvals and other authorisations or notifications as required will be provided and maintained on file.
- Transfer of micro-organism materials between the consortium partners will be facilitated using Material Transfer Agreements. As part of the Grant Agreement all participants will declare intellectual property relevant to the project and consent to the preparation of royalty free licence agreements between consortium members for the transfer of materials between the project partners. Details on the materials, transferred, imported to/exported from the EU will be kept on file. Copies of import/export authorisations, as required by national/EU legislation will be kept on file.
  - Import of micro-organism materials from Serbia or China into the EU and or export of micro-organism materials from the EU to Serbia or China will be licenced as required and copies of any import and export licences will be kept on file. Further information about the possible harm to the environment caused by the research and the measures that will be taken to mitigate the risks will be kept on file. Documentation demonstrating compliance with the UN Convention on Biological Diversity will be provided
- In addition to the BioICEP research activity being accepted and complying with the legal obligations of the non-EU countries, the activities will also be confirmed to be allowed in at least one EU Member State of the BioICEP consortium. The consortium partners will confirm this condition is met as part of the grant agreement
- In the case that researchers travel to work in Serbia or China a risk assessment will be undertaken taking appropriate safety measures into account. In case activities undertaken in Serbia and China raise ethics issues, the BioICEP consortium partners will ensure that the research conducted outside the EU is legal in at least one EU Member State.

## Ethics Procedures

### Transboundary Movement of Biological agents

Laboratories carrying out a purely diagnostic service are not required to notify the Authority unless they are working with a group 4 biological agent. However, if the laboratory is deliberately propagating or concentrating group 2 or group 3 biological agents, then notification will be required.

Notification may be made within Ireland using the [biological agents' notification form](#) or by other suitable methods which are jurisdiction specific within the EU. If using an alternative method, it is a legal requirement that the information as detailed in Regulation 14 (1) (f) of the [Biological Agents' Regulations](#) within Ireland, or equivalent regulations within other EU countries is included in the notification.

### Transboundary Movement of GMO's

Any GMOs developed within the project will be contained. The required procedures are provided by the Irish Environmental Protection Agency <https://www.epa.ie/licensing/gmo/> for the transboundary movement of GMO's. This procedure is outlined as follows:

#### Notification requirements for the first time use of a premises for a Class 1 GMM:

- An assessment of the risks to human health and the environment associated with the contained use activity (refer to [Third Schedule](#) of the [GMO \(Contained Use\) Regulations, 2001 to 2010](#)). A [sample Class 1 GMM RA](#) may be referenced on the EPA website);
- Information relating to waste management;
- Information as set out under Part A of the 5<sup>th</sup> Schedule of the aforementioned Regulations (please find form attached);
- Fee of €250 (EPA bank details attached).

#### Notification requirements for the first time use of a premises for the contained use of a Class 2 GMM:

- A Risk Assessment (RA) (refer to Article 13 of the GMO (Contained Use) Regulations, 2001 to 2010. A [sample Class 2 GMM RA](#) may be referenced on the EPA website);
- Information relating to waste management;
- Information as set out under Part A of the 5<sup>th</sup> Schedule of the aforementioned Regulations (please find form attached);
- Fee of €1,875 (EPA bank details attached).

Articles 12(1) and 12(3) of Regulations (EC) No 1946/2003 on transboundary movement of GMOs state

1. Exporters shall ensure that the following information is stated in a document accompanying the GMO and is transmitted to the importer receiving the GMO:

- (a) that it contains or consists of GMOs;
- (b) the unique identification code(s) assigned to those GMOs if such codes exist.

2. For GMOs intended for contained use, the information referred to in paragraph 1 shall be supplemented by a declaration by the exporter which shall specify:

(a) any requirements for the safe handling, storage, transport and use of these GMOs;

(b) the contact point for further information, including the name and address of the individual or institution to whom or which the GMOs are consigned

Equivalent notification requirements available in other EU countries can also be applied.

**In the case that regulatory obstacles occur on the import or export of plastics or micro-organism materials between Serbia and or China and the EU, the following measures will be implemented to insure that the achievement of the project objectives is not impeded.**

The project will use transfer of people between Serbia and China and the EU, which is already an integral part of the project plan, to ensure that tasks can be completed and repeated in each of the relevant jurisdictions. Protocols will be developed and implemented by the same people, both in China and the EU and Serbia, ensuring the best performing materials and processes are developed and established simultaneously in both China and the EU and Serbia. Full transparency on data, materials and processes is of fundamental importance to the project and the complete reproducibility of performance in both China and the EU and Serbia will be verified at regular intervals, by teams of people traveling between these countries. This validation process will be fully documented and maintained on file.

In addition to this the team at AIT continue to pursue the resolution of import or export of plastics or micro-organism materials between Serbia and or China and the EU. The Chinese authorities have confirmed that currently materials other than genetically modified organisms can be transferred with agreement of the courier companies. This will fulfill the requirements for the first stage of the project which involves the transfer of non-GMO waste plastic materials available in the open environment. These can be contained within the laboratories and prepared for courier transfer to the EU and or Serbia. In order to achieve the resolution of GMO materials, AIT are working with national bodies such as the Department of Agriculture Food and Marine and the National Environmental Protection Agency with progress expected within the coming months and the duration of the project.

The ability to successfully achieve the project tasks, however does not depend on the access to the passage of materials between China and the EU and or Serbia. The straight forward passage of materials will only serve to reinforce the technologies developed rather than present an obstacle to their development. As stated the strong two-way people transfer policy between China and the EU and or Serbia is the primary means to accomplishing the project tasks and objectives

## 5.2 Security

No.	Question:	Answer:
1.	Will the BioCEP project involve activities or results raising security issues?	No
2.	Will the BioCEP project involve 'EU-classified information' as background or results:	No

ESTIMATED BUDGET FOR THE ACTION

	Estimated eligible <sup>1</sup> costs (per budget category)									EU contribution			Additional information			
	A. Direct personnel costs				B. Direct costs of subcontracting	<i>[C. Direct costs of fin. support]</i>	D. Other direct costs		E. Indirect costs <sup>2</sup>	Total costs	Reimbursement rate %	Maximum EU contribution <sup>3</sup>	Maximum grant amount <sup>4</sup>	Information for indirect costs	Information for auditors	Other information:
	A.1 Employees (or equivalent)		A.4 SME owners without salary				D.1 Travel	D.5 Costs of internally invoiced goods and services						Estimated costs of in-kind contributions not used on premises	Declaration of costs under Point D.4	Estimated costs of beneficiaries/ linked third parties not receiving funding/ international partners
	A.2 Natural persons under direct contract		A.5 Beneficiaries that are natural persons without salary				D.2 Equipment									
A.3 Seconded persons				D.3 Other goods and services												
Form of costs <sup>6</sup>	Actual	Unit <sup>7</sup>	Unit <sup>8</sup>		Actual	Actual	Actual	Unit <sup>9</sup>	Flat-rate <sup>10</sup>							
									25%							
	a	Total b	No hours	Total c	d	<i>[e]</i>	f	Total g	h = 0,25 x (a +b+c+f+g + <i>[i1]</i> <sup>13</sup> + <i>[i2]</i> <sup>13</sup> -n)	j = a+b+c+d + <i>[e]</i> +f+g+h + <i>[i1]</i> + <i>[i2]</i>	k	l	m	n	Yes/No	
1. AIT	589 900.00	0.00	0.00	0.00	0.00	0.00	134 610.00	0.00	181 127.50	905 637.50	100.00	905 637.50	905 637.00	0.00	No	n/a
- IMCAS	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	635 641.00
- SU	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	635 641.00
- BIT	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	635 641.00
Total beneficiary	589 900.00	0.00			0.00	0.00	134 610.00	0.00	181 127.50	905 637.50		905 637.50	905 637.00	n/a	n/a	1 906 923.00
2. ACTECO	123 950.00	0.00	0.00	0.00	0.00	0.00	30 000.00	0.00	38 487.50	192 437.50	100.00	192 437.50	192 437.00	0.00	No	n/a
3. AIMPLAS	303 900.00	0.00	0.00	0.00	0.00	0.00	95 200.00	0.00	99 775.00	498 875.00	100.00	498 875.00	498 875.00	0.00	No	n/a
4. AVECOM	218 800.00	0.00	0.00	0.00	0.00	0.00	92 200.00	0.00	77 750.00	388 750.00	100.00	388 750.00	388 750.00	0.00	No	n/a
5. TUC	151 940.00	0.00	0.00	0.00	0.00	0.00	46 200.00	0.00	49 535.00	247 675.00	100.00	247 675.00	247 675.00	0.00	No	n/a
6. IMGGE	226 000.00	0.00	0.00	0.00	0.00	0.00	152 800.00	0.00	94 700.00	473 500.00	100.00	473 500.00	473 500.00	0.00	No	n/a
7. IBET	192 100.00	0.00	0.00	0.00	0.00	0.00	137 700.00	0.00	82 450.00	412 250.00	100.00	412 250.00	412 250.00	0.00	No	n/a
8. LIT	276 228.00	0.00	0.00	0.00	0.00	0.00	42 900.00	0.00	79 782.00	398 910.00	100.00	398 910.00	398 910.00	0.00	No	n/a
9. LOGOPLASTE	183 265.00	0.00	0.00	0.00	0.00	0.00	36 786.00	0.00	55 012.75	275 063.75	100.00	275 063.75	275 063.00	0.00	No	n/a
10. MicroLife	230 000.00	0.00	0.00	0.00	0.00	0.00	87 500.00	0.00	79 375.00	396 875.00	100.00	396 875.00	396 875.00	0.00	No	n/a
11. NTUA	196 212.00	0.00	0.00	0.00	0.00	0.00	110 200.00	0.00	76 603.00	383 015.00	100.00	383 015.00	383 015.00	0.00	No	n/a
12. TCD	263 097.00	0.00	0.00	0.00	0.00	0.00	76 745.00	0.00	84 960.50	424 802.50	100.00	424 802.50	424 802.00	0.00	No	n/a
Total consortium	2 955 392.00	0.00		0.00	0.00	0.00	1 042 841.00	0.00	999 558.25	4 997 791.25		4 997 791.25	4 997 789.00			1 906 923.00

<sup>1</sup> See Article 6 for the eligibility conditions.

<sup>2</sup> Indirect costs already covered by an operating grant (received under any EU or Euratom funding programme; see Article 6.5.(b)) are ineligible under the GA. Therefore, a beneficiary/linked third party that receives an operating grant during the action's duration cannot declare indirect costs for the year(s)/reporting period(s) covered by the operating grant, unless it can demonstrate that the operating grant does not cover any costs of the action (see Article 6.2.E).

<sup>3</sup> This is the theoretical amount of EU contribution that the system calculates automatically (by multiplying all the budgeted costs by the reimbursement rate). This theoretical amount is capped by the 'maximum grant amount' (that the Commission decided to grant for the action) (see Article 5.1).

<sup>4</sup> The 'maximum grant amount' is the maximum grant amount decided by the Commission. It normally corresponds to the requested grant, but may be lower.

<sup>5</sup> Depending on its type, this specific cost category will or will not cover indirect costs. Specific unit costs that include indirect costs are: costs for energy efficiency measures in buildings, access costs for providing trans-national access to research infrastructure and costs for clinical studies.

<sup>6</sup> See Article 5 for the forms of costs.

<sup>7</sup> Unit : hours worked on the action; costs per unit (hourly rate) : calculated according to the beneficiary's usual accounting practice.

<sup>8</sup> See Annex 2a 'Additional information on the estimated budget' for the details (costs per hour (hourly rate)).

<sup>9</sup> Unit and costs per unit : calculated according to the beneficiary's usual accounting practices.

<sup>10</sup> Flat rate : 25% of eligible direct costs, from which are excluded: direct costs of subcontracting, costs of in-kind contributions not used on premises, direct costs of financial support, and unit costs declared under budget category F if they include indirect costs (see Article 6.2.E).

<sup>11</sup> See Annex 2a 'Additional information on the estimated budget' for the details (units, costs per unit).

<sup>12</sup> See Annex 2a 'Additional information on the estimated budget' for the details (units, costs per unit, estimated number of units, etc).

<sup>13</sup> Only specific unit costs that do not include indirect costs.

<sup>14</sup> See Article 9 for beneficiaries not receiving funding.

<sup>15</sup> Only for linked third parties that receive funding.

**ANNEX 2a****ADDITIONAL INFORMATION ON THE ESTIMATED BUDGET**

- Instructions and footnotes in blue will not appear in the text generated by the IT system (since they are internal instructions only).
- For options [in square brackets]: the applicable option will be chosen by the IT system. Options not chosen will automatically not appear.
- For fields in [grey in square brackets] (even if they are part of an option as specified in the previous item): IT system will enter the appropriate data.

**⚠ Transitory period:** Until SyGMA fully supports Annex 2a, you must prepare it manually (using this template by choosing and deleting the options/entering the appropriate data).  
 For the 'unit cost tables': either fill them out manually or use currently existing tables from Annex 1 or the proposal.  
 The document can then be uploaded in SyGMA and attached to the grant agreement.

**Unit cost for SME owners/natural beneficiaries without salary****1. Costs for a [SME owner//beneficiary that is a natural person] not receiving a salary**

Units: hours worked on the action

Amount per unit ('hourly rate'): calculated according to the following formula:

{the monthly living allowance for researchers in MSCA-IF actions / 143 hours}  
 multiplied by  
 {country-specific correction coefficient of the country where the beneficiary is established}

The monthly living allowance and the country-specific correction coefficients are set out in the Work Programme (section 3 MSCA) in force at the time of the call:

- for calls *before* Work Programme 2018-2020:
  - for the monthly living allowance: **EUR 4 650**
  - for the country-specific correction coefficients: see Work Programme 2014-2015 and Work Programme 2016-2017 (available on the [Participant Portal Reference Documents](#) page)
- for calls *under* Work Programme 2018-2020:
  - for the monthly living allowance: **EUR 4 880**
  - for the country-specific correction coefficients: see Work Programme 2018-2020 (available on the [Participant Portal Reference Documents](#) page)

**[additional OPTION for beneficiaries/linked third parties that have opted to use the unit cost (in the proposal/with an amendment):** For the following beneficiaries/linked third parties, the amounts per unit (hourly rate) are fixed as follows:

- beneficiary/linked third party [short name]: EUR [insert amount]
  - beneficiary/linked third party [short name]: EUR [insert amount]
- [same for other beneficiaries/linked third parties, if necessary] ]

Estimated number of units: see Annex 2

## **Energy efficiency measures unit cost**

### **2. Costs for energy efficiency measures in buildings**

Unit: m<sup>2</sup> of eligible ‘conditioned’ (i.e. built or refurbished) floor area

Amount per unit\*: see (for each beneficiary/linked third party and BEST table) the ‘unit cost table’ attached

\* Amount calculated as follows:  
{EUR 0.1 x estimated total kWh saved per m<sup>2</sup> per year x 10}

Estimated number of units: see (for each beneficiary/linked third party and BEST table) the ‘unit cost table’ attached

Unit cost table (energy efficiency measures unit cost)<sup>1</sup>

Short name beneficiary/linked third party	BEST No	Amount per unit	Estimated No of units	Total unit cost (cost per unit x estimated no of units)

---

<sup>1</sup> Data from the ‘building energy specification table (BEST)’ that is part of the proposal and Annex 1.



## **Research infrastructure unit cost**

### **3. Access costs for providing trans-national access to research infrastructure**

Units<sup>2</sup>: see (for each access provider and installation) the ‘unit cost table’ attached

Amount per unit<sup>\*</sup>: see (for each access provider and installation) the ‘unit cost table’ attached

\* Amount calculated as follows:

$$\frac{\text{average annual total access cost to the installation (over past two years)}^3}{\text{average annual total quantity of access to the installation (over past two years)}^4}$$

Estimated number of units: see (for each access provider and installation) the ‘unit cost table’ attached

Unit cost table (access to research infrastructure unit cost)<sup>5</sup>

Short name access provider	Short name infrastru cture	Installation		Unit of access	Amount per unit	Estimated No of units	Total unit cost (cost per unit x estimated no of units)
		No	Short name				

## **Clinical studies unit cost**

### **4. Costs for clinical studies**

Units: patients/subjects that participate in the clinical study

Amount per unit<sup>\*</sup>: see (for each sequence (if any), clinical study and beneficiary/linked third party) the ‘unit cost table’ attached

\* Amount calculated, for the cost components of each task, as follows:

For **personnel costs**:

For personnel costs of doctors: ‘average hourly cost for doctors’, i.e.:

{certified or auditable total personnel costs for doctors for year N-1

{ 1720 \* number of full-time-equivalent for doctors for year N-1 }

multiplied by

estimated number of hours to be worked by doctors for the task (per participant)}

For personnel costs of other medical personnel: ‘average hourly cost for other medical personnel’, i.e.:

{certified or auditable total personnel costs for other medical personnel for year N-1

{ 1720 \* number of full-time-equivalent for other medical personnel for year N-1 }

<sup>2</sup> Unit of access (e.g. beam hours, weeks of access, sample analysis) fixed by the access provider in proposal.

<sup>3</sup> In exceptional and duly justified cases, the Commission/Agency may agree to a different reference period.

<sup>4</sup> In exceptional and duly justified cases, the Commission/Agency may agree to a different reference period.

<sup>5</sup> Data from the ‘table on estimated costs/quantity of access to be provided’ that is part of the proposal and Annex 1.

**H2020 Templates: Annex 2a (Additional information on the estimated budget)**

multiplied by  
estimated number of hours to be worked by other medical personnel for the task (per participant))

For personnel costs of technical personnel: 'average hourly cost for technical personnel', i.e.:

$$\frac{\{\text{certified or auditable total personnel costs for technical personnel for year N-1}\}}{\{1720 * \text{number of full-time-equivalent for technical personnel for year N-1}\}}$$

multiplied by  
estimated number of hours to be worked by technical personnel for the task (per participant))

'total personnel costs' means actual salaries + actual social security contributions + actual taxes and other costs included in the remuneration, provided they arise from national law or the employment contract/equivalent appointing act

For **consumables**:

For each cost item: 'average price of the consumable', i.e.:

$$\frac{\{\{\text{certified or auditable total costs of purchase of the consumable in year N-1}\}}{\text{total number of items purchased in year N-1}\}}$$

multiplied by  
estimated number of items to be used for the task (per participant))

'total costs of purchase of the consumable' means total value of the supply contracts (including related duties, taxes and charges such as non-deductible VAT) concluded by the beneficiary for the consumable delivered in year N-1, provided the contracts were awarded according to the principle of best value- for-money and without any conflict of interests

For **medical equipment**:

For each cost item: 'average cost of depreciation and directly related services per unit of use', i.e.:

$$\frac{\{\{\text{certified or auditable total depreciation costs in year N-1} + \text{certified or auditable total costs of purchase of services in year N-1 for the category of equipment concerned}\}}{\text{total capacity in year N-1}}$$

multiplied by  
estimated number of units of use of the equipment for the task (per participant))

'total depreciation costs' means total depreciation allowances as recorded in the beneficiary's accounts of year N-1 for the category of equipment concerned, provided the equipment was purchased according to the principle of best value for money and without any conflict of interests + total costs of renting or leasing contracts (including related duties, taxes and charges such as non-deductible VAT) in year N-1 for the category of equipment concerned, provided they do not exceed the depreciation costs of similar equipment and do not include finance fees

For **services**:

For each cost item: 'average cost of the service per study participant', i.e.:

$$\frac{\{\text{certified or auditable total costs of purchase of the service in year N-1}\}}{\text{total number of patients or subjects included in the clinical studies for which the service was delivered in year N-1}\}}$$

'total costs of purchase of the service' means total value of the contracts concluded by the beneficiary (including related duties, taxes and charges such as non-deductible VAT) for the specific service delivered in year N-1 for the conduct of clinical studies, provided the contracts were awarded according to the principle of best value for money and without any conflict of interests

For **indirect costs**:

$$\{\{\{\text{cost component 'personnel costs'} + \text{cost component 'consumables'} + \text{cost component 'medical equipment'}\}\}$$

minus

$$\{\text{costs of in-kind contributions provided by third parties which are not used on the beneficiary's premises} + \text{costs of providing financial support to third parties (if any)}\}$$

multiplied by

$$25\% \}$$

**H2020 Templates: Annex 2a (Additional information on the estimated budget)**

The estimation of the resources to be used must be done on the basis of the study protocol and must be the same for all beneficiaries/linked third parties/third parties involved.

The year N-1 to be used is the last closed financial year at the time of submission of the grant application.

Estimated number of units: see (for each clinical study and beneficiary/linked third party) the 'unit cost table' attached

Unit cost table: clinical studies unit cost<sup>6</sup>

Task, Direct cost categories	Resource per patient	Costs year N-1 Beneficiary 1 [short name]	Costs year N-1 Linked third party 1a [short name]	Costs year N-1 Beneficiary 2 [short name]	Costs year N-1 Linked third party 2a [short name]	Costs year N-1 Third party giving in-kind contributions 1 [short name]
<b><u>Sequence No. 1</u></b>						
<b>Task No. 1</b> Blood sample						
(a) <b>Personnel costs:</b> - Doctors	n/a					
- Other Medical Personnel	Phlebotomy (nurse), 10 minutes	8,33 EUR	11,59 EUR	10,30 EUR	11,00 EUR	9,49 EUR
- Technical Personnel	Sample Processing (lab technician), 15 minutes	9,51 EUR	15,68 EUR	14,60 EUR	15,23 EUR	10,78 EUR
(b) <b>Costs of consumables:</b>	Syringe	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	Cannula	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	Blood container	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(c) <b>Costs of medical equipment:</b>	Use of -80° deep freezer, 60 days	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	Use of centrifuge, 15 minutes	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(d) <b>Costs of services</b>	Cleaning of XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(e) <b>Indirect costs (25% flat-rate)</b>		XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
<b>Task No. 2</b>						
...						
<b>Amount per unit (unit cost sequence 1):</b>		XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
<b><u>Sequence No. 2</u></b>						
<b>Task No. 1</b>						

<sup>6</sup> Same table as in proposal and Annex 1.

## H2020 Templates: Annex 2a (Additional information on the estimated budget)

XXX						
(a) <b>Personnel costs:</b>						
- Doctors	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
- Other Medical Personnel	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
- Technical Personnel	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(b) <b>Costs of consumables:</b>	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(c) <b>Costs of medical equipment:</b>	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(d) <b>Costs of services</b>	XXX	XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
(e) <b>Indirect costs</b> (25% flat-rate)		XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
<b>Task No. 2</b>						
...						
<b>Amount per unit (unit cost sequence 2):</b>		XX EUR	XX EUR	XX EUR	XX EUR	XX EUR
...						
<b>Amount per unit (unit cost entire study):</b>		XX EUR	XX EUR	XX EUR	XX EUR	XX EUR

## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**ACTECO PRODUCTOS Y SERVICIOS SL (ACTECO)**, established in C ZAMORA 24 POLIGONO INDUSTRIAL L ALFAC III, IBI ALICANTE 03440, Spain, VAT number: ESB03971512, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary** No ('2')

**in Grant Agreement No** 870292 ('the Agreement')

**between** ATHLONE INSTITUTE OF TECHNOLOGY **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**AIMPLAS - ASOCIACION DE INVESTIGACION DE MATERIALES PLASTICOS Y CONEXAS (AIMPLAS)**, established in CALLE GUSTAVE EIFFEL 4 PARQUE TECNOLOGICO DE PATERNA, PATERNA VALENCIA 46980, Spain, VAT number: ESG46714853, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary** No ('3')

**in Grant Agreement** No 870292 ('the Agreement')

**between** ATHLONE INSTITUTE OF TECHNOLOGY **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary



## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**AVECOM (AVECOM)**, established in INDUSTRIEWEG 122P, GENT-WONDELGEM 9032, Belgium, VAT number: BE0454894069, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary** No ('4')

**in Grant Agreement No** 870292 ('the Agreement')

**between** ATHLONE INSTITUTE OF TECHNOLOGY **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**TECHNISCHE UNIVERSITÄT CLAUSTHAL (TUC)**, established in ADOLPH ROMER STRASSE 2A, CLAUSTHAL ZELLERFELD 38678, Germany, VAT number: DE811282802, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary No ('5')**

**in Grant Agreement No 870292 ('the Agreement')**

**between** ATHLONE INSTITUTE OF TECHNOLOGY **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**INSTITUT ZA MOLEKULARNU GENETIKU I GENETICKO INZENJERSTVO (IMGGE)**, established in VOJVODE STEPE 444A, BEOGRAD 11010, Serbia, VAT number: RS101736673, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary** No ('6')

**in Grant Agreement** No 870292 ('the Agreement')

**between** ATHLONE INSTITUTE OF TECHNOLOGY **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**INSTITUTO DE BIOLOGIA EXPERIMENTAL E TECNOLOGICA (IBET)**, established in AVENIDA DA REPUBLICA QUINTO DO MARQUES, OEIRAS 2781 901, Portugal, VAT number: PT502112255, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary** No ('7')

**in Grant Agreement No** 870292 ('the Agreement')

**between** ATHLONE INSTITUTE OF TECHNOLOGY **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**LIMERICK INSTITUTE OF TECHNOLOGY (LIT)**, established in MOYLISH PARK, LIMERICK, Ireland, VAT number: IE6609432C, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary** No ('8')

**in Grant Agreement No** 870292 ('the Agreement')

**between** **ATHLONE INSTITUTE OF TECHNOLOGY** **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**LOGOPLASTE INNOVATION LAB LDA (LOGOPLASTE)**, established in ESTRADA DA MALVEIRA ED LOGOPLASTE MATO ROMAO, CASCAIS 2750 782, Portugal, VAT number: PT505323354, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary** No ('9')

**in Grant Agreement** No 870292 ('the Agreement')

**between** ATHLONE INSTITUTE OF TECHNOLOGY **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary



## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**MICROLIFE SOLUTIONS BV (MicroLife)**, established in SCIENCE PARK 406, AMSTERDAM 1098 XH, Netherlands, VAT number: NL850870938B01, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary** No ('10')

**in Grant Agreement No** 870292 ('the Agreement')

**between** ATHLONE INSTITUTE OF TECHNOLOGY **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**NATIONAL TECHNICAL UNIVERSITY OF ATHENS - NTUA (NTUA)**, established in HEROON POLYTECHNIOU 9 ZOGRAPHOU CAMPUS, ATHINA 15780, Greece, VAT number: EL099793475, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary** No ('11')

**in Grant Agreement No** 870292 ('the Agreement')

**between** ATHLONE INSTITUTE OF TECHNOLOGY **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

## ANNEX 3

### ACCESSION FORM FOR BENEFICIARIES

**THE PROVOST, FELLOWS, FOUNDATION SCHOLARS & THE OTHER MEMBERS OF BOARD OF THE COLLEGE OF THE HOLY & UNDIVIDED TRINITY OF QUEEN ELIZABETH NEAR DUBLIN (TCD)**, established in College Green, DUBLIN 2, Ireland, VAT number: IE2200007U, ('the beneficiary'), represented for the purpose of signing this Accession Form by the undersigned,

**hereby agrees**

**to become beneficiary** No ('12')

**in Grant Agreement No 870292** ('the Agreement')

**between** **ATHLONE INSTITUTE OF TECHNOLOGY** **and** the European Union ('the EU'), represented by the European Commission ('the Commission'),

**for the action entitled** 'Bio Innovation of a Circular Economy for Plastics (BioICEP)'.

**and mandates**

**the coordinator** to submit and sign in its name and on its behalf any **amendments** to the Agreement, in accordance with Article 55.

By signing this Accession Form, the beneficiary accepts the grant and agrees to implement it in accordance with the Agreement, with all the obligations and conditions it sets out.

SIGNATURE

For the beneficiary

FINANCIAL STATEMENT FOR [BENEFICIARY [name]/ LINKED THIRD PARTY [name]] FOR REPORTING PERIOD [reporting period]

	Eligible <sup>1</sup> costs (per budget category)													Receipts	EU contribution			Additional information	
	A. Direct personnel costs			B. Direct costs of subcontracting	[C. Direct costs of fin. support]	D. Other direct costs		E. Indirect costs <sup>2</sup>	[F. Costs of ... ]		Total costs	Receipts	Reimburse ment rate %	Maximum EU contribution <sup>3</sup>	Requested EU contribution	Information for indirect costs :			
	A.1 Employees (or equivalent)	A.4 SME owners without salary		[C.1 Financial support]	D.1 Travel	[D.4 Costs of large research infrastructure]	D.5 Costs of internally invoiced goods and services		[F.1 Costs of ...]	[F.2 Costs of ...]		Receipts of the action, to be reported in the last reporting period, according to Article 5.3.3				Costs of in-kind contributions not used on premises			
	A.2 Natural persons under direct contract	A.5 Beneficiaries that are natural persons without salary		[C.2 Prizes]	D.2 Equipment														
A.3 Seconded persons  [A.6 Personnel for providing access to research infrastructure]				D.3 Other goods and services															
Form of costs <sup>4</sup>	Actual	Unit	Unit		Actual	Actual	Actual	Actual	Unit	Flat-rate <sup>5</sup>		Unit	[Unit][Lump sum]						
										25%									
	a	Total b	No hours	Total c	d	[e]	f	[g]	Total h	i=0,25 x (a+b+c+f+[g] + h+ [j 1] <sup>6</sup> +[j2] <sup>6</sup> -p)	No units	Total [j1]	Total [j2]	k = a+b+c+d+[e] +f+ [g] +h+ i + [j1] +[j2]	l	m	n	o	p
[short name beneficiary/linked third party]																			

**The beneficiary/linked third party hereby confirms that:**  
The information provided is complete, reliable and true.  
The costs declared are eligible (see Article 6).  
The costs can be substantiated by adequate records and supporting documentation that will be produced upon request or in the context of checks, reviews, audits and investigations (see Articles 17, 18 and 22).  
For the last reporting period: that all the receipts have been declared (see Article 5.3.3).

📌 Please declare all eligible costs, even if they exceed the amounts indicated in the estimated budget (see Annex 2). Only amounts that were declared in your individual financial statements can be taken into account lateron, in order to replace other costs that are found to be ineligible.

<sup>1</sup> See Article 6 for the eligibility conditions

<sup>2</sup> The indirect costs claimed must be free of any amounts covered by an operating grant (received under any EU or Euratom funding programme; see Article 6.2.E). If you have received an operating grant during this reporting period, you cannot claim indirect costs unless you can demonstrate that the operating grant does not cover any costs of the action.

<sup>3</sup> This is the *theoretical* amount of EU contribution that the system calculates automatically (by multiplying the reimbursement rate by the total costs declared). The amount you request (in the column 'requested EU contribution') may be less,

<sup>4</sup> See Article 5 for the forms of costs

<sup>5</sup> Flat rate : 25% of eligible direct costs, from which are excluded: direct costs of subcontracting, costs of in-kind contributions not used on premises, direct costs of financial support, and unit costs declared under budget category F if they include indirect costs (see Article 6.2.E)

<sup>6</sup> Only specific unit costs that do not include indirect costs

## ANNEX 5

### MODEL FOR THE CERTIFICATE ON THE FINANCIAL STATEMENTS

- For options [*in italics in square brackets*]: choose the applicable option. Options not chosen should be deleted.
- For fields in [grey in square brackets]: enter the appropriate data

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TERMS OF REFERENCE FOR AN INDEPENDENT REPORT OF FACTUAL FINDINGS ON COSTS DECLARED UNDER A GRANT AGREEMENT FINANCED UNDER THE HORIZON 2020 RESEARCH FRAMEWORK PROGRAMME

INDEPENDENT REPORT OF FACTUAL FINDINGS ON COSTS DECLARED UNDER A GRANT AGREEMENT FINANCED UNDER THE HORIZON 2020 RESEARCH FRAMEWORK PROGRAMME

## **Terms of Reference for an Independent Report of Factual Findings on costs declared under a Grant Agreement financed under the Horizon 2020 Research and Innovation Framework Programme**

This document sets out the ‘**Terms of Reference (ToR)**’ under which

[OPTION 1: [insert name of the beneficiary] (*‘the Beneficiary’*)] [OPTION 2: [insert name of the linked third party] (*‘the Linked Third Party’*), third party linked to the Beneficiary [insert name of the beneficiary] (*‘the Beneficiary’*)]

agrees to engage

[insert legal name of the auditor] (*‘the Auditor’*)

to produce an independent report of factual findings (*‘the Report’*) concerning the Financial Statement(s)<sup>1</sup> drawn up by the [Beneficiary] [Linked Third Party] for the Horizon 2020 grant agreement [insert number of the grant agreement, title of the action, acronym and duration from/to] (*‘the Agreement’*), and

to issue a Certificate on the Financial Statements’ (*‘CFS’*) referred to in Article 20.4 of the Agreement based on the compulsory reporting template stipulated by the Commission.

The Agreement has been concluded under the Horizon 2020 Research and Innovation Framework Programme (H2020) between the Beneficiary and [OPTION 1: *the European Union, represented by the European Commission (‘the Commission’)*][OPTION 2: *the European Atomic Energy Community (Euratom,) represented by the European Commission (‘the Commission’)*][OPTION 3: *the [Research Executive Agency (REA)] [European Research Council Executive Agency (ERCEA)] [Innovation and Networks Executive Agency (INEA)] [Executive Agency for Small and Medium-sized Enterprises (EASME)] (‘the Agency’), under the powers delegated by the European Commission (‘the Commission’).*]

The [Commission] [Agency] is mentioned as a signatory of the Agreement with the Beneficiary only. The [European Union][Euratom][Agency] is not a party to this engagement.

### **1.1 Subject of the engagement**

The coordinator must submit to the [Commission][Agency] the final report within 60 days following the end of the last reporting period which should include, amongst other documents, a CFS for each beneficiary and for each linked third party that requests a total contribution of EUR 325 000 or more, as reimbursement of actual costs and unit costs calculated on the basis of its usual cost accounting practices (see Article 20.4 of the Agreement). The CFS must cover all reporting periods of the beneficiary or linked third party indicated above.

The Beneficiary must submit to the coordinator the CFS for itself and for its linked third party(ies), if the CFS must be included in the final report according to Article 20.4 of the Agreement.

The CFS is composed of two separate documents:

- The Terms of Reference (*‘the ToR’*) to be signed by the [Beneficiary] [Linked Third Party] and the Auditor;

---

<sup>1</sup> By which costs under the Agreement are declared (see template ‘Model Financial Statements’ in Annex 4 to the Grant Agreement).



- The Auditor's Independent Report of Factual Findings ('the Report') to be issued on the Auditor's letterhead, dated, stamped and signed by the Auditor (or the competent public officer) which includes the agreed-upon procedures ('the Procedures') to be performed by the Auditor, and the standard factual findings ('the Findings') to be confirmed by the Auditor.

If the CFS must be included in the final report according to Article 20.4 of the Agreement, the request for payment of the balance relating to the Agreement cannot be made without the CFS. However, the payment for reimbursement of costs covered by the CFS does not preclude the Commission [ Agency,] the European Anti-Fraud Office and the European Court of Auditors from carrying out checks, reviews, audits and investigations in accordance with Article 22 of the Agreement.

## 1.2 Responsibilities

The [Beneficiary] [Linked Third Party]:

- must draw up the Financial Statement(s) for the action financed by the Agreement in compliance with the obligations under the Agreement. The Financial Statement(s) must be drawn up according to the [Beneficiary's] [Linked Third Party's] accounting and book-keeping system and the underlying accounts and records;
- must send the Financial Statement(s) to the Auditor;
- is responsible and liable for the accuracy of the Financial Statement(s);
- is responsible for the completeness and accuracy of the information provided to enable the Auditor to carry out the Procedures. It must provide the Auditor with a written representation letter supporting these statements. The written representation letter must state the period covered by the statements and must be dated;
- accepts that the Auditor cannot carry out the Procedures unless it is given full access to the [Beneficiary's] [Linked Third Party's] staff and accounting as well as any other relevant records and documentation.

The Auditor:

- [Option 1 by default: is qualified to carry out statutory audits of accounting documents in accordance with Directive 2006/43/EC of the European Parliament and of the Council of 17 May 2006 on statutory audits of annual accounts and consolidated accounts, amending Council Directives 78/660/EEC and 83/349/EEC and repealing Council Directive 84/253/EEC or similar national regulations].
- [Option 2 if the Beneficiary or Linked Third Party has an independent Public Officer: is a competent and independent Public Officer for which the relevant national authorities have established the legal capacity to audit the Beneficiary].
- [Option 3 if the Beneficiary or Linked Third Party is an international organisation: is an [internal] [external] auditor in accordance with the internal financial regulations and procedures of the international organisation].

The Auditor:

- must be independent from the Beneficiary [and the Linked Third Party], in particular, it must not have been involved in preparing the [Beneficiary's] [Linked Third Party's] Financial Statement(s);
- must plan work so that the Procedures may be carried out and the Findings may be assessed;
- must adhere to the Procedures laid down and the compulsory report format;
- must carry out the engagement in accordance with this ToR;
- must document matters which are important to support the Report;
- must base its Report on the evidence gathered;
- must submit the Report to the [Beneficiary] [Linked Third Party].

The Commission sets out the Procedures to be carried out by the Auditor. The Auditor is not responsible for their suitability or pertinence. As this engagement is not an assurance engagement, the Auditor does not provide an audit opinion or a statement of assurance.

### 1.3 Applicable Standards

The Auditor must comply with these Terms of Reference and with<sup>2</sup>:

- the International Standard on Related Services ('ISRS') 4400 *Engagements to perform Agreed-upon Procedures regarding Financial Information* as issued by the International Auditing and Assurance Standards Board (IAASB);
- the *Code of Ethics for Professional Accountants* issued by the International Ethics Standards Board for Accountants (IESBA). Although ISRS 4400 states that independence is not a requirement for engagements to carry out agreed-upon procedures, the [Commission]/[Agency] requires that the Auditor also complies with the Code's independence requirements.

The Auditor's Report must state that there is no conflict of interests in establishing this Report between the Auditor and the Beneficiary *[and the Linked Third Party]*, and must specify - if the service is invoiced - the total fee paid to the Auditor for providing the Report.

### 1.4 Reporting

The Report must be written in the language of the Agreement (see Article 20.7).

Under Article 22 of the Agreement, the Commission[, the Agency], the European Anti-Fraud Office and the Court of Auditors have the right to audit any work that is carried out under the action and for which costs are declared from [the European Union] [Euratom] budget. This includes work related to this engagement. The Auditor must provide access to all working papers (e.g. recalculation of hourly rates, verification of the time declared for the action) related to this assignment if the Commission [, the Agency], the European Anti-Fraud Office or the European Court of Auditors requests them.

### 1.5 Timing

The Report must be provided by [dd Month yyyy].

### 1.6 Other terms

*[The [Beneficiary] [Linked Third Party] and the Auditor can use this section to agree other specific terms, such as the Auditor's fees, liability, applicable law, etc. Those specific terms must not contradict the terms specified above.]*

[legal name of the Auditor]	[legal name of the [Beneficiary]/[Linked Third Party]]
[name & function of authorised representative]	[name & function of authorised representative]
[dd Month yyyy]	[dd Month yyyy]
Signature of the Auditor	Signature of the [Beneficiary]/[Linked Third Party]

<sup>2</sup> Supreme Audit Institutions applying INTOSAI-standards may carry out the Procedures according to the corresponding International Standards of Supreme Audit Institutions and code of ethics issued by INTOSAI instead of the International Standard on Related Services ('ISRS') 4400 and the Code of Ethics for Professional Accountants issued by the IAASB and the IESBA.

**Independent Report of Factual Findings on costs declared  
under Horizon 2020 Research and Innovation Framework Programme**

*(To be printed on the Auditor's letterhead)*

To  
[ name of contact person(s)], [Position]  
[ [Beneficiary's] [Linked Third Party's] name ]  
[ Address]  
[ dd Month yyyy]

Dear [Name of contact person(s)],

As agreed under the terms of reference dated [dd Month yyyy]

with [OPTION 1: [insert name of the beneficiary] ('the Beneficiary')] [OPTION 2: [insert name of the linked third party] ('the Linked Third Party'), third party linked to the Beneficiary [insert name of the beneficiary] ('the Beneficiary')],

we

[name of the auditor] ('the Auditor'),  
established at  
[full address/city/state/province/country],  
represented by  
[name and function of an authorised representative],

have carried out the procedures agreed with you regarding the costs declared in the Financial Statement(s)<sup>3</sup> of the [Beneficiary] [Linked Third Party] concerning the grant agreement [insert grant agreement reference: number, title of the action and acronym] ('the Agreement'),

with a total cost declared of  
[total amount] EUR,

and a total of actual costs and unit costs calculated in accordance with the [Beneficiary's] [Linked Third Party's] usual cost accounting practices' declared of

[sum of total actual costs and total direct personnel costs declared as unit costs calculated in accordance with the [Beneficiary's] [Linked Third Party's] usual cost accounting practices] EUR

and **hereby provide our Independent Report of Factual Findings ('the Report')** using the compulsory report format agreed with you.

**The Report**

Our engagement was carried out in accordance with the terms of reference ('the ToR') appended to this Report. The Report includes the agreed-upon procedures ('the Procedures') carried out and the standard factual findings ('the Findings') examined.

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<sup>3</sup> By which the Beneficiary declares costs under the Agreement (see template 'Model Financial Statement' in Annex 4 to the Agreement).

The Procedures were carried out solely to assist the [Commission] [Agency] in evaluating whether the [Beneficiary's] [Linked Third Party's] costs in the accompanying Financial Statement(s) were declared in accordance with the Agreement. The [Commission] [Agency] draws its own conclusions from the Report and any additional information it may require.

The scope of the Procedures was defined by the Commission. Therefore, the Auditor is not responsible for their suitability or pertinence. Since the Procedures carried out constitute neither an audit nor a review made in accordance with International Standards on Auditing or International Standards on Review Engagements, the Auditor does not give a statement of assurance on the Financial Statements.

Had the Auditor carried out additional procedures or an audit of the [Beneficiary's] [Linked Third Party's] Financial Statements in accordance with International Standards on Auditing or International Standards on Review Engagements, other matters might have come to its attention and would have been included in the Report.

### **Not applicable Findings**

We examined the Financial Statement(s) stated above and considered the following Findings not applicable:

*Explanation (to be removed from the Report):*

*If a Finding was not applicable, it must be marked as 'N.A.' ('Not applicable') in the corresponding row on the right-hand column of the table and means that the Finding did not have to be corroborated by the Auditor and the related Procedure(s) did not have to be carried out.*

*The reasons of the non-application of a certain Finding must be obvious i.e.*

- i) if no cost was declared under a certain category then the related Finding(s) and Procedure(s) are not applicable;*
- ii) if the condition set to apply certain Procedure(s) are not met the related Finding(s) and those Procedure(s) are not applicable. For instance, for 'beneficiaries with accounts established in a currency other than euro' the Procedure and Finding related to 'beneficiaries with accounts established in euro' are not applicable. Similarly, if no additional remuneration is paid, the related Finding(s) and Procedure(s) for additional remuneration are not applicable.*

**List here all Findings considered not applicable for the present engagement and explain the reasons of the non-applicability.**

....

### **Exceptions**

Apart from the exceptions listed below, the [Beneficiary] [Linked Third Party] provided the Auditor all the documentation and accounting information needed by the Auditor to carry out the requested Procedures and evaluate the Findings.

*Explanation (to be removed from the Report):*

- If the Auditor was not able to successfully complete a procedure requested, it must be marked as 'E' ('Exception') in the corresponding row on the right-hand column of the table. The reason such as the inability to reconcile key information or the unavailability of data that prevents the Auditor from carrying out the Procedure must be indicated below.*
- If the Auditor cannot corroborate a standard finding after having carried out the corresponding procedure, it must also be marked as 'E' ('Exception') and, where possible, the reasons why the Finding was not fulfilled and its possible impact must be explained here below.*

**List here any exceptions and add any information on the cause and possible consequences of each exception, if known. If the exception is quantifiable, include the corresponding amount.**

....

**Example (to be removed from the Report):**

1. *The Beneficiary was unable to substantiate the Finding number 1 on ... because ....*
2. *Finding number 30 was not fulfilled because the methodology used by the Beneficiary to calculate unit costs was different from the one approved by the Commission. The differences were as follows: ...*
3. *After carrying out the agreed procedures to confirm the Finding number 31, the Auditor found a difference of \_\_\_\_\_ EUR. The difference can be explained by ...*

**Further Remarks**

In addition to reporting on the results of the specific procedures carried out, the Auditor would like to make the following general remarks:

**Example (to be removed from the Report):**

1. *Regarding Finding number 8 the conditions for additional remuneration were considered as fulfilled because ...*
2. *In order to be able to confirm the Finding number 15 we carried out the following additional procedures: ....*

**Use of this Report**

This Report may be used only for the purpose described in the above objective. It was prepared solely for the confidential use of the [Beneficiary] [Linked Third Party] and the [Commission] [Agency], and only to be submitted to the [Commission] [Agency] in connection with the requirements set out in Article 20.4 of the Agreement. The Report may not be used by the [Beneficiary] [Linked Third Party] or by the [Commission] [Agency] for any other purpose, nor may it be distributed to any other parties. The [Commission] [Agency] may only disclose the Report to authorised parties, in particular to the European Anti-Fraud Office (OLAF) and the European Court of Auditors.

This Report relates only to the Financial Statement(s) submitted to the [Commission] [Agency] by the [Beneficiary] [Linked Third Party] for the Agreement. Therefore, it does not extend to any other of the [Beneficiary's] [Linked Third Party's] Financial Statement(s).

There was no conflict of interest<sup>4</sup> between the Auditor and the Beneficiary [and Linked Third Party] in establishing this Report. The total fee paid to the Auditor for providing the Report was EUR [ ] (including EUR [ ] of deductible VAT).

We look forward to discussing our Report with you and would be pleased to provide any further information or assistance.

[legal name of the Auditor]

[name and function of an authorised representative]

[dd Month yyyy]

Signature of the Auditor

<sup>4</sup> A conflict of interest arises when the Auditor's objectivity to establish the certificate is compromised in fact or in appearance when the Auditor for instance:

- was involved in the preparation of the Financial Statements;
- stands to benefit directly should the certificate be accepted;
- has a close relationship with any person representing the beneficiary;
- is a director, trustee or partner of the beneficiary; or
- is in any other situation that compromises his or her independence or ability to establish the certificate impartially.

**Agreed-upon procedures to be performed and standard factual findings to be confirmed by the Auditor**

The European Commission reserves the right to i) provide the auditor with additional guidance regarding the procedures to be followed or the facts to be ascertained and the way in which to present them (this may include sample coverage and findings) or to ii) change the procedures, by notifying the Beneficiary in writing. The procedures carried out by the auditor to confirm the standard factual finding are listed in the table below.

If this certificate relates to a Linked Third Party, any reference here below to ‘the Beneficiary’ is to be considered as a reference to ‘the Linked Third Party’.

The ‘result’ column has three different options: ‘C’, ‘E’ and ‘N.A.’:

- ‘C’ stands for ‘confirmed’ and means that the auditor can confirm the ‘standard factual finding’ and, therefore, there is no exception to be reported.
- ‘E’ stands for ‘exception’ and means that the Auditor carried out the procedures but cannot confirm the ‘standard factual finding’, or that the Auditor was not able to carry out a specific procedure (e.g. because it was impossible to reconcile key information or data were unavailable),
- ‘N.A.’ stands for ‘not applicable’ and means that the Finding did not have to be examined by the Auditor and the related Procedure(s) did not have to be carried out. The reasons of the non-application of a certain Finding must be obvious i.e. i) if no cost was declared under a certain category then the related Finding(s) and Procedure(s) are not applicable; ii) if the condition set to apply certain Procedure(s) are not met then the related Finding(s) and Procedure(s) are not applicable. For instance, for ‘beneficiaries with accounts established in a currency other than the euro’ the Procedure related to ‘beneficiaries with accounts established in euro’ is not applicable. Similarly, if no additional remuneration is paid, the related Finding(s) and Procedure(s) for additional remuneration are not applicable.

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
A	<b>ACTUAL PERSONNEL COSTS AND UNIT COSTS CALCULATED BY THE BENEFICIARY IN ACCORDANCE WITH ITS USUAL COST ACCOUNTING PRACTICE</b>		
	<p>The Auditor draws a sample of persons whose costs were declared in the Financial Statement(s) to carry out the procedures indicated in the consecutive points of this section A.</p> <p><i>(The sample should be selected randomly so that it is representative. Full coverage is required if there are fewer than 10 people (including employees, natural persons working under a direct contract and personnel seconded by a third party), otherwise the sample should have a minimum of 10 people, or 10% of the total, whichever number is the highest)</i></p> <p>The Auditor sampled [ ] people out of the total of [ ] people.</p>		



Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
A.1	<p><b>PERSONNEL COSTS</b></p> <p><u>For the persons included in the sample and working under an employment contract or equivalent act (general procedures for individual actual personnel costs and personnel costs declared as unit costs)</u></p> <p>To confirm standard factual findings 1-5 listed in the next column, the Auditor reviewed following information/documents provided by the Beneficiary:</p> <ul style="list-style-type: none"> <li>○ a list of the persons included in the sample indicating the period(s) during which they worked for the action, their position (classification or category) and type of contract;</li> <li>○ the payslips of the employees included in the sample;</li> <li>○ reconciliation of the personnel costs declared in the Financial Statement(s) with the accounting system (project accounting and general ledger) and payroll system;</li> <li>○ information concerning the employment status and employment conditions of personnel included in the sample, in particular their employment contracts or equivalent;</li> <li>○ the Beneficiary's usual policy regarding payroll matters (e.g. salary policy, overtime policy, variable pay);</li> <li>○ applicable national law on taxes, labour and social security and</li> <li>○ any other document that supports the personnel costs declared.</li> </ul> <p>The Auditor also verified the eligibility of all components of the retribution (see Article 6 GA) and recalculated the personnel costs for employees included in the sample.</p>	1) The employees were i) directly hired by the Beneficiary in accordance with its national legislation, ii) under the Beneficiary's sole technical supervision and responsibility and iii) remunerated in accordance with the Beneficiary's usual practices.	
		2) Personnel costs were recorded in the Beneficiary's accounts/payroll system.	
		3) Costs were adequately supported and reconciled with the accounts and payroll records.	
		4) Personnel costs did not contain any ineligible elements.	
		5) There were no discrepancies between the personnel costs charged to the action and the costs recalculated by the Auditor.	
	<p><i>Further procedures if 'additional remuneration' is paid</i></p> <p>To confirm standard factual findings 6-9 listed in the next column, the Auditor:</p> <ul style="list-style-type: none"> <li>○ reviewed relevant documents provided by the Beneficiary (legal form, legal/statutory</li> </ul>	6) The Beneficiary paying "additional remuneration" was a non-profit legal entity.	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	<p>obligations, the Beneficiary's usual policy on additional remuneration, criteria used for its calculation, the Beneficiary's usual remuneration practice for projects funded under national funding schemes...);</p> <ul style="list-style-type: none"> <li>recalculated the amount of additional remuneration eligible for the action based on the supporting documents received (full-time or part-time work, exclusive or non-exclusive dedication to the action, usual remuneration paid for projects funded by national schemes) to arrive at the applicable FTE/year and pro-rata rate (see data collected in the course of carrying out the procedures under A.2 'Productive hours' and A.4 'Time recording system').</li> </ul> <p><i>'ADDITIONAL REMUNERATION' MEANS ANY PART OF THE REMUNERATION WHICH EXCEEDS WHAT THE PERSON WOULD BE PAID FOR TIME WORKED IN PROJECTS FUNDED BY NATIONAL SCHEMES.</i></p> <p><i>IF ANY PART OF THE REMUNERATION PAID TO THE EMPLOYEE QUALIFIES AS "ADDITIONAL REMUNERATION" AND IS ELIGIBLE UNDER THE PROVISIONS OF ARTICLE 6.2.A.1, THIS CAN BE CHARGED AS ELIGIBLE COST TO THE ACTION UP TO THE FOLLOWING AMOUNT:</i></p> <p>(A) <i>IF THE PERSON WORKS FULL TIME AND EXCLUSIVELY ON THE ACTION DURING THE FULL YEAR: UP TO EUR 8 000/YEAR;</i></p> <p>(B) <i>IF THE PERSON WORKS EXCLUSIVELY ON THE ACTION BUT NOT FULL-TIME OR NOT FOR THE FULL YEAR: UP TO THE CORRESPONDING PRO-RATA AMOUNT OF EUR 8 000, OR</i></p> <p>(C) <i>IF THE PERSON DOES NOT WORK EXCLUSIVELY ON THE ACTION: UP TO A PRO-RATA AMOUNT CALCULATED IN ACCORDANCE TO ARTICLE 6.2.A.1.</i></p>	<p>7) The amount of additional remuneration paid corresponded to the Beneficiary's usual remuneration practices and was consistently paid whenever the same kind of work or expertise was required.</p>	
		<p>8) The criteria used to calculate the additional remuneration were objective and generally applied by the Beneficiary regardless of the source of funding used.</p>	
		<p>9) The amount of additional remuneration included in the personnel costs charged to the action was capped at EUR 8,000 per FTE/year (up to the equivalent pro-rata amount if the person did not work on the action full-time during the year or did not work exclusively on the action).</p>	
	<p><i>Additional procedures in case "unit costs calculated by the Beneficiary in accordance with its usual cost accounting practices" is applied:</i></p> <p>Apart from carrying out the procedures indicated above to confirm standard factual findings 1-5 and, if applicable, also 6-9, the Auditor carried out following procedures to confirm standard</p>	<p>10) The personnel costs included in the Financial Statement were calculated in accordance with the Beneficiary's usual cost accounting practice. This methodology was consistently</p>	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	<p>factual findings 10-13 listed in the next column:</p> <ul style="list-style-type: none"> <li>obtained a description of the Beneficiary's usual cost accounting practice to calculate unit costs;</li> <li>reviewed whether the Beneficiary's usual cost accounting practice was applied for the Financial Statements subject of the present CFS;</li> <li>verified the employees included in the sample were charged under the correct category (in accordance with the criteria used by the Beneficiary to establish personnel categories) by reviewing the contract/HR-record or analytical accounting records;</li> <li>verified that there is no difference between the total amount of personnel costs used in calculating the cost per unit and the total amount of personnel costs recorded in the statutory accounts;</li> <li>verified whether actual personnel costs were adjusted on the basis of budgeted or estimated elements and, if so, verified whether those elements used are actually relevant for the calculation, objective and supported by documents.</li> </ul>	used in all H2020 actions.	
		11) The employees were charged under the correct category.	
		12) Total personnel costs used in calculating the unit costs were consistent with the expenses recorded in the statutory accounts.	
		13) Any estimated or budgeted element used by the Beneficiary in its unit-cost calculation were relevant for calculating personnel costs and corresponded to objective and verifiable information.	
	<p><u>For natural persons included in the sample and working with the Beneficiary under a direct contract other than an employment contract, such as consultants (no subcontractors).</u></p> <p>To confirm standard factual findings 14-17 listed in the next column the Auditor reviewed following information/documents provided by the Beneficiary:</p> <ul style="list-style-type: none"> <li>the contracts, especially the cost, contract duration, work description, place of work, ownership of the results and reporting obligations to the Beneficiary;</li> <li>the employment conditions of staff in the same category to compare costs and;</li> <li>any other document that supports the costs declared and its registration (e.g. invoices, accounting records, etc.).</li> </ul>	14) The natural persons worked under conditions similar to those of an employee, in particular regarding the way the work is organised, the tasks that are performed and the premises where they are performed.	
		15) The results of work carried out belong to the Beneficiary, or, if not, the Beneficiary has obtained all necessary rights to fulfil its obligations as if those	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
		results were generated by itself.	
		16) Their costs were not significantly different from those for staff who performed similar tasks under an employment contract with the Beneficiary.	
		17) The costs were supported by audit evidence and registered in the accounts.	
	<p><u>For personnel seconded by a third party and included in the sample (not subcontractors)</u></p> <p>To confirm standard factual findings 18-21 listed in the next column, the Auditor reviewed following information/documents provided by the Beneficiary:</p> <ul style="list-style-type: none"> <li>○ their secondment contract(s) notably regarding costs, duration, work description, place of work and ownership of the results;</li> <li>○ if there is reimbursement by the Beneficiary to the third party for the resource made available (in-kind contribution against payment): any documentation that supports the costs declared (e.g. contract, invoice, bank payment, and proof of registration in its accounting/payroll, etc.) and reconciliation of the Financial Statement(s) with the accounting system (project accounting and general ledger) as well as any proof that the amount invoiced by the third party did not include any profit;</li> <li>○ if there is no reimbursement by the Beneficiary to the third party for the resource made available (in-kind contribution free of charge): a proof of the actual cost borne by the Third Party for the resource made available free of charge to the Beneficiary such as a statement of costs incurred by the Third Party and proof of the registration in the Third Party's accounting/payroll;</li> </ul>	18) Seconded personnel reported to the Beneficiary and worked on the Beneficiary's premises (unless otherwise agreed with the Beneficiary).	
		19) The results of work carried out belong to the Beneficiary, or, if not, the Beneficiary has obtained all necessary rights to fulfil its obligations as if those results were generated by itself..	
		<p><i>If personnel is seconded against payment:</i></p> <p>20) The costs declared were supported with documentation and recorded in the</p>	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	<ul style="list-style-type: none"> <li>any other document that supports the costs declared (e.g. invoices, etc.).</li> </ul>	Beneficiary's accounts. The third party did not include any profit.	
		<i>If personnel is seconded free of charge:</i>  21) The costs declared did not exceed the third party's cost as recorded in the accounts of the third party and were supported with documentation.	
A.2	<b>PRODUCTIVE HOURS</b>  To confirm standard factual findings 22-27 listed in the next column, the Auditor reviewed relevant documents, especially national legislation, labour agreements and contracts and time records of the persons included in the sample, to verify that: <ul style="list-style-type: none"> <li>the annual productive hours applied were calculated in accordance with one of the methods described below,</li> <li>the full-time equivalent (FTEs) ratios for employees not working full-time were correctly calculated.</li> </ul> If the Beneficiary applied method B, the auditor verified that the correctness in which the total number of hours worked was calculated and that the contracts specified the annual workable hours.  If the Beneficiary applied method C, the auditor verified that the 'annual productive hours' applied when calculating the hourly rate were equivalent to at least 90 % of the 'standard annual workable hours'. The Auditor can only do this if the calculation of the standard annual workable	22) The Beneficiary applied method [choose one option and delete the others]  [A: 1720 hours]  [B: the 'total number of hours worked']  [C: 'standard annual productive hours' used correspond to usual accounting practices]	
		23) Productive hours were calculated annually.	
		24) For employees not working full-time the full-time equivalent (FTE) ratio was correctly applied.	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	<p>hours can be supported by records, such as national legislation, labour agreements, and contracts.</p> <p><i>BENEFICIARY'S PRODUCTIVE HOURS' FOR PERSONS WORKING FULL TIME SHALL BE ONE OF THE FOLLOWING METHODS:</i></p> <p><i>A. 1720 ANNUAL PRODUCTIVE HOURS (PRO-RATA FOR PERSONS NOT WORKING FULL-TIME)</i></p> <p><i>B. THE TOTAL NUMBER OF HOURS WORKED BY THE PERSON FOR THE BENEFICIARY IN THE YEAR (THIS METHOD IS ALSO REFERRED TO AS 'TOTAL NUMBER OF HOURS WORKED' IN THE NEXT COLUMN). THE CALCULATION OF THE TOTAL NUMBER OF HOURS WORKED WAS DONE AS FOLLOWS: ANNUAL WORKABLE HOURS OF THE PERSON ACCORDING TO THE EMPLOYMENT CONTRACT, APPLICABLE LABOUR AGREEMENT OR NATIONAL LAW PLUS OVERTIME WORKED MINUS ABSENCES (SUCH AS SICK LEAVE OR SPECIAL LEAVE).</i></p> <p><i>C. THE STANDARD NUMBER OF ANNUAL HOURS GENERALLY APPLIED BY THE BENEFICIARY FOR ITS PERSONNEL IN ACCORDANCE WITH ITS USUAL COST ACCOUNTING PRACTICES (THIS METHOD IS ALSO REFERRED TO AS 'STANDARD ANNUAL PRODUCTIVE HOURS' IN THE NEXT COLUMN). THIS NUMBER MUST BE AT LEAST 90% OF THE STANDARD ANNUAL WORKABLE HOURS.</i></p> <p><i>'ANNUAL WORKABLE HOURS' MEANS THE PERIOD DURING WHICH THE PERSONNEL MUST BE WORKING, AT THE EMPLOYER'S DISPOSAL AND CARRYING OUT HIS/HER ACTIVITY OR DUTIES UNDER THE EMPLOYMENT CONTRACT, APPLICABLE COLLECTIVE LABOUR AGREEMENT OR NATIONAL WORKING TIME LEGISLATION.</i></p>	<p><i>If the Beneficiary applied method B.</i></p> <p>25) The calculation of the number of 'annual workable hours', overtime and absences was verifiable based on the documents provided by the Beneficiary.</p> <p>25.1) The Beneficiary calculates the hourly rates per full financial year following procedure A.3 (method B is not allowed for beneficiaries calculating hourly rates per month).</p> <p><i>If the Beneficiary applied method C.</i></p> <p>26) The calculation of the number of 'standard annual workable hours' was verifiable based on the documents provided by the Beneficiary.</p>	



Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
		27) The ‘annual productive hours’ used for calculating the hourly rate were consistent with the usual cost accounting practices of the Beneficiary and were equivalent to at least 90 % of the ‘annual workable hours’.	
A.3	<p><b>HOURLY PERSONNEL RATES</b></p> <p><u>I) For unit costs calculated in accordance to the Beneficiary's usual cost accounting practice (unit costs):</u></p> <p>If the Beneficiary has a "Certificate on Methodology to calculate unit costs " (CoMUC) approved by the Commission, the Beneficiary provides the Auditor with a description of the approved methodology and the Commission’s letter of acceptance. The Auditor verified that the Beneficiary has indeed used the methodology approved. If so, no further verification is necessary.</p> <p>If the Beneficiary does not have a "Certificate on Methodology" (CoMUC) approved by the Commission, or if the methodology approved was not applied, then the Auditor:</p> <ul style="list-style-type: none"> <li>○ reviewed the documentation provided by the Beneficiary, including manuals and internal guidelines that explain how to calculate hourly rates;</li> <li>○ recalculated the unit costs (hourly rates) of staff included in the sample following the results of the procedures carried out in A.1 and A.2.</li> </ul> <p><u>II) For individual hourly rates:</u></p> <p>The Auditor:</p> <ul style="list-style-type: none"> <li>○ reviewed the documentation provided by the Beneficiary, including manuals and internal guidelines that explain how to calculate hourly rates;</li> </ul>	<p>28) The Beneficiary applied [<i>choose one option and delete the other</i>]:</p> <p>[Option I: “Unit costs (hourly rates) were calculated in accordance with the Beneficiary’s usual cost accounting practices”]</p> <p>[Option II: Individual hourly rates were applied]</p> <p><i>For option I concerning unit costs and if the Beneficiary applies the methodology approved by the Commission (CoMUC):</i></p> <p>29) The Beneficiary used the Commission-approved methodology to calculate hourly rates. It corresponded to the organisation's usual cost accounting practices and was applied consistently for all</p>	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	<ul style="list-style-type: none"> <li>recalculated the hourly rates of staff included in the sample (recalculation of all hourly rates if the Beneficiary uses annual rates, recalculation of three months selected randomly for every year and person if the Beneficiary uses monthly rates) following the results of the procedures carried out in A.1 and A.2;</li> <li>(only in case of monthly rates) confirmed that the time spent on parental leave is not deducted, and that, if parts of the basic remuneration are generated over a period longer than a month, the Beneficiary has included only the share which is generated in the month.</li> </ul>	activities irrespective of the source of funding.	
	<p><u>“UNIT COSTS CALCULATED BY THE BENEFICIARY IN ACCORDANCE WITH ITS USUAL COST ACCOUNTING PRACTICES”:</u></p> <p><i>IT IS CALCULATED BY DIVIDING THE TOTAL AMOUNT OF PERSONNEL COSTS OF THE CATEGORY TO WHICH THE EMPLOYEE BELONGS VERIFIED IN LINE WITH PROCEDURE A.1 BY THE NUMBER OF FTE AND THE ANNUAL TOTAL PRODUCTIVE HOURS OF THE SAME CATEGORY CALCULATED BY THE BENEFICIARY IN ACCORDANCE WITH PROCEDURE A.2.</i></p> <p><u>HOURLY RATE FOR INDIVIDUAL ACTUAL PERSONAL COSTS:</u></p> <p><i>IT IS CALCULATED FOLLOWING ONE OF THE TWO OPTIONS BELOW:</i></p> <p><i>A) [OPTION BY DEFAULT] BY DIVIDING THE ACTUAL ANNUAL AMOUNT OF PERSONNEL COSTS OF AN EMPLOYEE VERIFIED IN LINE WITH PROCEDURE A.1 BY THE NUMBER OF ANNUAL PRODUCTIVE HOURS VERIFIED IN LINE WITH PROCEDURE A.2 (FULL FINANCIAL YEAR HOURLY RATE);</i></p> <p><i>B) BY DIVIDING THE ACTUAL MONTHLY AMOUNT OF PERSONNEL COSTS OF AN EMPLOYEE VERIFIED IN LINE WITH PROCEDURE A.1 BY 1/12 OF THE NUMBER OF ANNUAL PRODUCTIVE HOURS VERIFIED IN LINE WITH PROCEDURE A.2.(MONTHLY HOURLY RATE).</i></p>	<p><i>For option I concerning unit costs and if the Beneficiary applies a methodology not approved by the Commission:</i></p> <p>30) The unit costs re-calculated by the Auditor were the same as the rates applied by the Beneficiary.</p>	
		<p><i>For option II concerning individual hourly rates:</i></p> <p>31) The individual rates re-calculated by the Auditor were the same as the rates applied by the Beneficiary.</p> <p>31.1) The Beneficiary used only one option (per full financial year or per month) throughout each financial year examined.</p> <p>31.2) The hourly rates do not include additional remuneration.</p>	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
A.4	<b>TIME RECORDING SYSTEM</b> To verify that the time recording system ensures the fulfilment of all minimum requirements and that the hours declared for the action were correct, accurate and properly authorised and supported by documentation, the Auditor made the following checks for the persons included in the sample that declare time as worked for the action on the basis of time records: <ul style="list-style-type: none"> <li>○ description of the time recording system provided by the Beneficiary (registration, authorisation, processing in the HR-system);</li> <li>○ its actual implementation;</li> <li>○ time records were signed at least monthly by the employees (on paper or electronically) and authorised by the project manager or another manager;</li> <li>○ the hours declared were worked within the project period;</li> <li>○ there were no hours declared as worked for the action if HR-records showed absence due to holidays or sickness (further cross-checks with travels are carried out in B.1 below) ;</li> <li>○ the hours charged to the action matched those in the time recording system.</li> </ul>	32) All persons recorded their time dedicated to the action on a <b>daily/ weekly/ monthly</b> basis using a <b>paper/computer-based</b> system. <i>(delete the answers that are not applicable)</i>	
		33) Their time-records were authorised at least monthly by the project manager or other superior.	
		34) Hours declared were worked within the project period and were consistent with the presences/absences recorded in HR-records.	
		35) There were no discrepancies between the number of hours charged to the action and the number of hours recorded.	
	<p><i>ONLY THE HOURS WORKED ON THE ACTION CAN BE CHARGED. ALL WORKING TIME TO BE CHARGED SHOULD BE RECORDED THROUGHOUT THE DURATION OF THE PROJECT, ADEQUATELY SUPPORTED BY EVIDENCE OF THEIR REALITY AND RELIABILITY (SEE SPECIFIC PROVISIONS BELOW FOR PERSONS WORKING EXCLUSIVELY FOR THE ACTION WITHOUT TIME RECORDS).</i></p> <p><u>If the persons are working exclusively for the action and without time records</u></p> <p>For the persons selected that worked exclusively for the action without time records, the Auditor verified evidence available demonstrating that they were in reality exclusively dedicated to the action and that the Beneficiary signed a declaration confirming that they have worked exclusively for the action.</p>	36) The exclusive dedication is supported by a declaration signed by the Beneficiary and by any other evidence gathered.	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
<b>B</b>	<b>COSTS OF SUBCONTRACTING</b>		
<b>B.1</b>	<p><b>The Auditor obtained the detail/breakdown of subcontracting costs and sampled cost items selected randomly</b> (full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest).</p> <p>To confirm standard factual findings 37-41 listed in the next column, the Auditor reviewed the following for the items included in the sample:</p> <ul style="list-style-type: none"> <li>○ the use of subcontractors was foreseen in Annex 1;</li> <li>○ subcontracting costs were declared in the subcontracting category of the Financial Statement;</li> <li>○ supporting documents on the selection and award procedure were followed;</li> <li>○ the Beneficiary ensured best value for money (key elements to appreciate the respect of this principle are the award of the subcontract to the bid offering best price-quality ratio, under conditions of transparency and equal treatment. In case an existing framework contract was used the Beneficiary ensured it was established on the basis of the principle of best value for money under conditions of transparency and equal treatment).</li> </ul> <p>In particular,</p> <ol style="list-style-type: none"> <li>i. if the Beneficiary acted as a contracting authority within the meaning of Directive 2004/18/EC (or 2014/24/EU) or of Directive 2004/17/EC (or 2014/25/EU), the Auditor verified that the applicable national law on public procurement was followed and that the subcontracting complied with the Terms and Conditions of the Agreement.</li> <li>ii. if the Beneficiary did not fall under the above-mentioned category the Auditor verified that the Beneficiary followed their usual procurement rules and respected the Terms and Conditions of the Agreement..</li> </ol>	<p>37) The use of claimed subcontracting costs was foreseen in Annex 1 and costs were declared in the Financial Statements under the subcontracting category.</p> <p>38) There were documents of requests to different providers, different offers and assessment of the offers before selection of the provider in line with internal procedures and procurement rules. Subcontracts were awarded in accordance with the principle of best value for money.</p> <p><i>(When different offers were not collected the Auditor explains the reasons provided by the Beneficiary under the caption “Exceptions” of the Report. The Commission will analyse this information to evaluate whether these costs might be accepted as eligible)</i></p> <p>39) The subcontracts were not awarded to other Beneficiaries</p>	

Ref	Procedures	Standard factual finding	Result (C / E / N.A.)
	<p>For the items included in the sample the Auditor also verified that:</p> <ul style="list-style-type: none"> <li>○ the subcontracts were not awarded to other Beneficiaries in the consortium;</li> <li>○ there were signed agreements between the Beneficiary and the subcontractor;</li> <li>○ there was evidence that the services were provided by subcontractor;</li> </ul>	of the consortium.	
		40) All subcontracts were supported by signed agreements between the Beneficiary and the subcontractor.	
		41) There was evidence that the services were provided by the subcontractors.	
<b>C</b>	<b>COSTS OF PROVIDING FINANCIAL SUPPORT TO THIRD PARTIES</b>		
<b>C.1</b>	<p><b>The Auditor obtained the detail/breakdown of the costs of providing financial support to third parties and sampled [ ] cost items selected randomly</b> <i>(full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest).</i></p> <p>The Auditor verified that the following minimum conditions were met:</p> <ul style="list-style-type: none"> <li>a) the maximum amount of financial support for each third party did not exceed EUR 60 000, unless explicitly mentioned in Annex 1;</li> <li>b) the financial support to third parties was agreed in Annex 1 of the Agreement and the other provisions on financial support to third parties included in Annex 1 were respected.</li> </ul>	42) All minimum conditions were met	

D	OTHER ACTUAL DIRECT COSTS		
D.1	<b>COSTS OF TRAVEL AND RELATED SUBSISTENCE ALLOWANCES</b>  <b>The Auditor sampled [ ] cost items selected randomly</b> ( <i>full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is the highest</i> ).  The Auditor inspected the sample and verified that: <ul style="list-style-type: none"> <li>○ travel and subsistence costs were consistent with the Beneficiary's usual policy for travel. In this context, the Beneficiary provided evidence of its normal policy for travel costs (e.g. use of first class tickets, reimbursement by the Beneficiary on the basis of actual costs, a lump sum or per diem) to enable the Auditor to compare the travel costs charged with this policy;</li> <li>○ travel costs are correctly identified and allocated to the action (e.g. trips are directly linked to the action) by reviewing relevant supporting documents such as minutes of meetings, workshops or conferences, their registration in the correct project account, their consistency with time records or with the dates/duration of the workshop/conference;</li> <li>○ no ineligible costs or excessive or reckless expenditure was declared (see Article 6.5 MGA).</li> </ul>	43) Costs were incurred, approved and reimbursed in line with the Beneficiary's usual policy for travels.	
		44) There was a link between the trip and the action.	
		45) The supporting documents were consistent with each other regarding subject of the trip, dates, duration and reconciled with time records and accounting.	
		46) No ineligible costs or excessive or reckless expenditure was declared.	
D.2	<b>DEPRECIATION COSTS FOR EQUIPMENT, INFRASTRUCTURE OR OTHER ASSETS</b>  <b>The Auditor sampled [ ] cost items selected randomly</b> ( <i>full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is the highest</i> ).  For “equipment, infrastructure or other assets” [from now on called “asset(s)”] selected in the sample the Auditor verified that: <ul style="list-style-type: none"> <li>○ the assets were acquired in conformity with the Beneficiary's internal guidelines and procedures;</li> </ul>	47) Procurement rules, principles and guides were followed.	
		48) There was a link between the grant agreement and the asset charged to the action.	
		49) The asset charged to the action was traceable to the accounting records and the underlying documents.	



	<ul style="list-style-type: none"> <li>○ they were correctly allocated to the action (with supporting documents such as delivery note invoice or any other proof demonstrating the link to the action)</li> <li>○ they were entered in the accounting system;</li> <li>○ the extent to which the assets were used for the action (as a percentage) was supported by reliable documentation (e.g. usage overview table);</li> </ul> <p>The Auditor recalculated the depreciation costs and verified that they were in line with the applicable rules in the Beneficiary's country and with the Beneficiary's usual accounting policy (e.g. depreciation calculated on the acquisition value).</p> <p>The Auditor verified that no ineligible costs such as deductible VAT, exchange rate losses, excessive or reckless expenditure were declared (see Article 6.5 GA).</p>	50) The depreciation method used to charge the asset to the action was in line with the applicable rules of the Beneficiary's country and the Beneficiary's usual accounting policy.	
		51) The amount charged corresponded to the actual usage for the action.	
		52) No ineligible costs or excessive or reckless expenditure were declared.	
<b>D.3</b>	<p><b>COSTS OF OTHER GOODS AND SERVICES</b></p> <p><b>The Auditor sampled [ ] cost items selected randomly</b> (<i>full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest</i>).</p> <p>For the purchase of goods, works or services included in the sample the Auditor verified that:</p> <ul style="list-style-type: none"> <li>○ the contracts did not cover tasks described in Annex 1;</li> <li>○ they were correctly identified, allocated to the proper action, entered in the accounting system (traceable to underlying documents such as purchase orders, invoices and accounting);</li> <li>○ the goods were not placed in the inventory of durable equipment;</li> <li>○ the costs charged to the action were accounted in line with the Beneficiary's usual accounting practices;</li> <li>○ no ineligible costs or excessive or reckless expenditure were declared (see Article 6 GA).</li> </ul> <p>In addition, the Auditor verified that these goods and services were acquired in conformity with</p>	53) Contracts for works or services did not cover tasks described in Annex 1.	
		54) Costs were allocated to the correct action and the goods were not placed in the inventory of durable equipment.	
		55) The costs were charged in line with the Beneficiary's accounting policy and were adequately supported.	
		56) No ineligible costs or excessive or reckless expenditure were declared. For internal invoices/charges only the cost element was charged, without any mark-ups.	

	<p>the Beneficiary's internal guidelines and procedures, in particular:</p> <ul style="list-style-type: none"> <li>○ if Beneficiary acted as a contracting authority within the meaning of Directive 2004/18/EC (or 2014/24/EU) or of Directive 2004/17/EC (or 2014/25/EU), the Auditor verified that the applicable national law on public procurement was followed and that the procurement contract complied with the Terms and Conditions of the Agreement.</li> <li>○ if the Beneficiary did not fall into the category above, the Auditor verified that the Beneficiary followed their usual procurement rules and respected the Terms and Conditions of the Agreement.</li> </ul> <p>For the items included in the sample the Auditor also verified that:</p> <ul style="list-style-type: none"> <li>○ the Beneficiary ensured best value for money (key elements to appreciate the respect of this principle are the award of the contract to the bid offering best price-quality ratio, under conditions of transparency and equal treatment. In case an existing framework contract was used the Auditor also verified that the Beneficiary ensured it was established on the basis of the principle of best value for money under conditions of transparency and equal treatment);</li> </ul> <p><i>SUCH GOODS AND SERVICES INCLUDE, FOR INSTANCE, CONSUMABLES AND SUPPLIES, DISSEMINATION (INCLUDING OPEN ACCESS), PROTECTION OF RESULTS, SPECIFIC EVALUATION OF THE ACTION IF IT IS REQUIRED BY THE AGREEMENT, CERTIFICATES ON THE FINANCIAL STATEMENTS IF THEY ARE REQUIRED BY THE AGREEMENT AND CERTIFICATES ON THE METHODOLOGY, TRANSLATIONS, REPRODUCTION.</i></p>	<p>57) Procurement rules, principles and guides were followed. There were documents of requests to different providers, different offers and assessment of the offers before selection of the provider in line with internal procedures and procurement rules. The purchases were made in accordance with the principle of best value for money.</p> <p><i>(When different offers were not collected the Auditor explains the reasons provided by the Beneficiary under the caption “Exceptions” of the Report. The Commission will analyse this information to evaluate whether these costs might be accepted as eligible)</i></p>	
<b>D.4</b>	<p><b>AGGREGATED CAPITALISED AND OPERATING COSTS OF RESEARCH INFRASTRUCTURE</b></p> <p>The Auditor ensured the existence of a positive ex-ante assessment (issued by the EC Services) of the cost accounting methodology of the Beneficiary allowing it to apply the guidelines on direct costing for large research infrastructures in Horizon 2020.</p>	<p>58) The costs declared as direct costs for Large Research Infrastructures (in the appropriate line of the Financial Statement) comply with the methodology described in the positive ex-ante assessment report.</p>	

	<p><b><i>In the cases that a positive ex-ante assessment has been issued (see the standard factual findings 58-59 on the next column),</i></b> The Auditor ensured that the beneficiary has applied consistently the methodology that is explained and approved in the positive ex ante assessment;</p> <p><b><i>In the cases that a positive ex-ante assessment has NOT been issued (see the standard factual findings 60 on the next column),</i></b> The Auditor verified that no costs of Large Research Infrastructure have been charged as direct costs in any costs category;</p> <p><b><i>In the cases that a draft ex-ante assessment report has been issued with recommendation for further changes (see the standard factual findings 60 on the next column),</i></b></p> <ul style="list-style-type: none"> <li>The Auditor followed the same procedure as above (when a positive ex-ante assessment has NOT yet been issued) and paid particular attention (testing reinforced) to the cost items for which the draft ex-ante assessment either rejected the inclusion as direct costs for Large Research Infrastructures or issued recommendations.</li> </ul>	59) Any difference between the methodology applied and the one positively assessed was extensively described and adjusted accordingly.	
		60) The direct costs declared were free from any indirect costs items related to the Large Research Infrastructure.	
<b>D.5</b>	<p><b>Costs of internally invoiced goods and services</b></p> <p><b>The Auditor sampled cost items selected randomly</b> (full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest).</p> <p>To confirm standard factual findings 61-65 listed in the next column, the Auditor:</p> <ul style="list-style-type: none"> <li>obtained a description of the Beneficiary's usual cost accounting practice to calculate costs of internally invoiced goods and services (unit costs);</li> <li>reviewed whether the Beneficiary's usual cost accounting practice was applied for the Financial Statements subject of the present CFS;</li> <li>ensured that the methodology to calculate unit costs is being used in a consistent manner, based on objective criteria, regardless of the source of funding;</li> <li>verified that any ineligible items or any costs claimed under other budget categories, in particular indirect costs, have not been taken into account when calculating the costs of</li> </ul>	61) The costs of internally invoiced goods and services included in the Financial Statement were calculated in accordance with the Beneficiary's usual cost accounting practice.	
		62) The cost accounting practices used to calculate the costs of internally invoiced goods and services were applied by the Beneficiary in a consistent manner based on objective criteria regardless of the source of funding.	
		63) The unit cost is calculated using the actual costs for the good or service recorded in the Beneficiary's accounts, excluding any ineligible cost or costs included in other	

	<p>internally invoiced goods and services (see Article 6 GA);</p> <ul style="list-style-type: none"> <li>o verified whether actual costs of internally invoiced goods and services were adjusted on the basis of budgeted or estimated elements and, if so, verified whether those elements used are actually relevant for the calculation, and correspond to objective and verifiable information.</li> <li>o verified that any costs of items which are not directly linked to the production of the invoiced goods or service (e.g. supporting services like cleaning, general accountancy, administrative support, etc. not directly used for production of the good or service) have not been taken into account when calculating the costs of internally invoiced goods and services.</li> <li>o verified that any costs of items used for calculating the costs internally invoiced goods and services are supported by audit evidence and registered in the accounts.</li> </ul>	<p>budget categories.</p>	
		64) The unit cost excludes any costs of items which are not directly linked to the production of the invoiced goods or service.	
		65) The costs items used for calculating the actual costs of internally invoiced goods and services were relevant, reasonable and correspond to objective and verifiable information.	
<b>E</b>	<b>USE OF EXCHANGE RATES</b>		
<b>E.1</b>	<p><u>a) For Beneficiaries with accounts established in a currency other than euros</u></p> <p><b>The Auditor sampled [ ] cost items selected randomly and verified that the exchange rates used for converting other currencies into euros were in accordance with the following rules established in the Agreement ( full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest):</b></p> <p><i>COSTS RECORDED IN THE ACCOUNTS IN A CURRENCY OTHER THAN EURO SHALL BE CONVERTED INTO EURO AT THE AVERAGE OF THE DAILY EXCHANGE RATES PUBLISHED IN THE C SERIES OF OFFICIAL JOURNAL OF THE EUROPEAN UNION (<a href="https://www.ecb.int/stats/exchange/eurofxref/html/index.en.html">https://www.ecb.int/stats/exchange/eurofxref/html/index.en.html</a> ), DETERMINED OVER THE CORRESPONDING REPORTING PERIOD.</i></p> <p><i>IF NO DAILY EURO EXCHANGE RATE IS PUBLISHED IN THE OFFICIAL JOURNAL OF THE EUROPEAN UNION FOR THE CURRENCY IN QUESTION, CONVERSION SHALL BE MADE AT THE AVERAGE OF THE MONTHLY ACCOUNTING RATES ESTABLISHED BY THE COMMISSION AND PUBLISHED ON ITS WEBSITE (<a href="http://ec.europa.eu/budget/contracts_grants/info_contracts/inforeuro/inforeuro_en.cfm">http://ec.europa.eu/budget/contracts_grants/info_contracts/inforeuro/inforeuro_en.cfm</a> ),</i></p>	<p>66) The exchange rates used to convert other currencies into Euros were in accordance with the rules established of the Grant Agreement and there was no difference in the final figures.</p>	

	DETERMINED OVER THE CORRESPONDING REPORTING PERIOD.		
	<p>b) For Beneficiaries with accounts established in euros</p> <p><b>The Auditor sampled [ ] cost items selected randomly and verified that the exchange rates used for converting other currencies into euros were in accordance with the following rules established in the Agreement</b> ( full coverage is required if there are fewer than 10 items, otherwise the sample should have a minimum of 10 item, or 10% of the total, whichever number is highest):</p> <p><i>COSTS INCURRED IN ANOTHER CURRENCY SHALL BE CONVERTED INTO EURO BY APPLYING THE BENEFICIARY'S USUAL ACCOUNTING PRACTICES.</i></p>	67) The Beneficiary applied its usual accounting practices.	

[legal name of the audit firm]

[name and function of an authorised representative]

[dd Month yyyy]

<Signature of the Auditor>

## ANNEX 6

### MODEL FOR THE CERTIFICATE ON THE METHODOLOGY

- For options [*in italics in square brackets*]: choose the applicable option. Options not chosen should be deleted.
- For fields in [grey in square brackets]: enter the appropriate data.

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TERMS OF REFERENCE FOR AN AUDIT ENGAGEMENT FOR A METHODOLOGY CERTIFICATE IN CONNECTION WITH ONE OR MORE GRANT AGREEMENTS FINANCED UNDER THE HORIZON 2020 RESEARCH AND INNOVATION FRAMEWORK PROGRAMME

INDEPENDENT REPORT OF FACTUAL FINDINGS ON THE METHODOLOGY CONCERNING GRANT AGREEMENTS FINANCED UNDER THE HORIZON 2020 RESEARCH AND INNOVATION FRAMEWORK PROGRAMME



**Terms of reference for an audit engagement for a methodology certificate  
in connection with one or more grant agreements financed  
under the Horizon 2020 Research and Innovation Framework Programme**

This document sets out the ‘**Terms of Reference (ToR)**’ under which

[OPTION 1: [insert name of the beneficiary] (*‘the Beneficiary’*)] [OPTION 2: [insert name of the linked third party] (*‘the Linked Third Party’*), third party linked to the Beneficiary [insert name of the beneficiary] (*‘the Beneficiary’*)]

agrees to engage

[insert legal name of the auditor] (*‘the Auditor’*)

to produce an independent report of factual findings (*‘the Report’*) concerning the [Beneficiary’s] [Linked Third Party’s] usual accounting practices for calculating and claiming direct personnel costs declared as unit costs (*‘the Methodology’*) in connection with grant agreements financed under the Horizon 2020 Research and Innovation Framework Programme.

The procedures to be carried out for the assessment of the methodology will be based on the grant agreement(s) detailed below:

[title and number of the grant agreement(s)] (*‘the Agreement(s)’*)

The Agreement(s) has(have) been concluded between the Beneficiary and [OPTION 1: *the European Union, represented by the European Commission (‘the Commission’)*][OPTION 2: *the European Atomic Energy Community (Euratom,) represented by the European Commission (‘the Commission’)*][OPTION 3: *the [Research Executive Agency (REA)] [European Research Council Executive Agency (ERCEA)] [Innovation and Networks Executive Agency (INEA)] [Executive Agency for Small and Medium-sized Enterprises (EASME)] (‘the Agency’), under the powers delegated by the European Commission (‘the Commission’).*].

The [Commission] [Agency] is mentioned as a signatory of the Agreement with the Beneficiary only. The [European Union] [Euratom] [Agency] is not a party to this engagement.

## **1.1 Subject of the engagement**

According to Article 18.1.2 of the Agreement, beneficiaries [and linked third parties] that declare direct personnel costs as unit costs calculated in accordance with their usual cost accounting practices may submit to the [Commission] [Agency], for approval, a certificate on the methodology (*‘CoMUC’*) stating that there are adequate records and documentation to prove that their cost accounting practices used comply with the conditions set out in Point A of Article 6.2.

The subject of this engagement is the CoMUC which is composed of two separate documents:

- the Terms of Reference (*‘the ToR’*) to be signed by the [Beneficiary] [Linked Third Party] and the Auditor;
- the Auditor’s Independent Report of Factual Findings (*‘the Report’*) issued on the Auditor’s letterhead, dated, stamped and signed by the Auditor which includes; the standard statements (*‘the Statements’*) evaluated and signed by the [Beneficiary] [Linked Third Party], the agreed-upon procedures (*‘the Procedures’*) performed by the Auditor and the standard factual findings

(‘the Findings’) assessed by the Auditor. The Statements, Procedures and Findings are summarised in the table that forms part of the Report.

The information provided through the Statements, the Procedures and the Findings will enable the Commission to draw conclusions regarding the existence of the *[Beneficiary’s]* *[Linked Third Party’s]* usual cost accounting practice and its suitability to ensure that direct personnel costs claimed on that basis comply with the provisions of the Agreement. The Commission draws its own conclusions from the Report and any additional information it may require.

## 1.2 Responsibilities

The parties to this agreement are the *[Beneficiary]* *[Linked Third Party]* and the Auditor.

The *[Beneficiary]* *[Linked Third Party]*:

- is responsible for preparing financial statements for the Agreement(s) (‘the Financial Statements’) in compliance with those Agreements;
- is responsible for providing the Financial Statement(s) to the Auditor and enabling the Auditor to reconcile them with the *[Beneficiary’s]* *[Linked Third Party’s]* accounting and bookkeeping system and the underlying accounts and records. The Financial Statement(s) will be used as a basis for the procedures which the Auditor will carry out under this ToR;
- is responsible for its Methodology and liable for the accuracy of the Financial Statement(s);
- is responsible for endorsing or refuting the Statements indicated under the heading ‘Statements to be made by the Beneficiary/ Linked Third Party’ in the first column of the table that forms part of the Report;
- must provide the Auditor with a signed and dated representation letter;
- accepts that the ability of the Auditor to carry out the Procedures effectively depends upon the *[Beneficiary]* *[Linked Third Party]* providing full and free access to the *[Beneficiary’s]* *[Linked Third Party’s]* staff and to its accounting and other relevant records.

The Auditor:

- *[Option 1 by default: is qualified to carry out statutory audits of accounting documents in accordance with Directive 2006/43/EC of the European Parliament and of the Council of 17 May 2006 on statutory audits of annual accounts and consolidated accounts, amending Council Directives 78/660/EEC and 83/349/EEC and repealing Council Directive 84/253/EEC or similar national regulations].*
- *[Option 2 if the Beneficiary or Linked Third Party has an independent Public Officer: is a competent and independent Public Officer for which the relevant national authorities have established the legal capacity to audit the Beneficiary].*
- *[Option 3 if the Beneficiary or Linked Third Party is an international organisation: is an [internal] [external] auditor in accordance with the internal financial regulations and procedures of the international organisation].*

The Auditor:

- must be independent from the Beneficiary *[and the Linked Third Party]*, in particular, it must not have been involved in preparing the Beneficiary’s *[and Linked Third Party’s]* Financial Statement(s);
- must plan work so that the Procedures may be carried out and the Findings may be assessed;
- must adhere to the Procedures laid down and the compulsory report format;
- must carry out the engagement in accordance with these ToR;
- must document matters which are important to support the Report;
- must base its Report on the evidence gathered;
- must submit the Report to the *[Beneficiary]* *[Linked Third Party]*.

The Commission sets out the Procedures to be carried out and the Findings to be endorsed by the Auditor. The Auditor is not responsible for their suitability or pertinence. As this engagement is not an assurance engagement the Auditor does not provide an audit opinion or a statement of assurance.

### 1.3 Applicable Standards

The Auditor must comply with these Terms of Reference and with<sup>1</sup>:

- the International Standard on Related Services ('ISRS') 4400 *Engagements to perform Agreed-upon Procedures regarding Financial Information* as issued by the International Auditing and Assurance Standards Board (IAASB);
- the *Code of Ethics for Professional Accountants* issued by the International Ethics Standards Board for Accountants (IESBA). Although ISRS 4400 states that independence is not a requirement for engagements to carry out agreed-upon procedures, the Commission requires that the Auditor also complies with the Code's independence requirements.

The Auditor's Report must state that there was no conflict of interests in establishing this Report between the Auditor and the Beneficiary *[and the Linked Third Party]* that could have a bearing on the Report, and must specify – if the service is invoiced - the total fee paid to the Auditor for providing the Report.

### 1.4 Reporting

The Report must be written in the language of the Agreement (see Article 20.7 of the Agreement).

Under Article 22 of the Agreement, the Commission, *[the Agency]*, the European Anti-Fraud Office and the Court of Auditors have the right to audit any work that is carried out under the action and for which costs are declared from *[the European Union]* *[Euratom]* budget. This includes work related to this engagement. The Auditor must provide access to all working papers related to this assignment if the Commission<sup>1</sup>, *the Agency*, the European Anti-Fraud Office or the European Court of Auditors requests them.

### 1.5 Timing

The Report must be provided by [dd Month yyyy].

### 1.6 Other Terms

*[The [Beneficiary] [Linked Third Party] and the Auditor can use this section to agree other specific terms, such as the Auditor's fees, liability, applicable law, etc. Those specific terms must not contradict the terms specified above.]*

[legal name of the Auditor]  
[name & title of authorised representative]  
[dd Month yyyy]  
Signature of the Auditor

[legal name of the [Beneficiary] [Linked Third Party]]  
[name & title of authorised representative]  
[dd Month yyyy]  
Signature of the *[Beneficiary]* *[Linked Third Party]*

<sup>1</sup> Supreme Audit Institutions applying INTOSAI-standards may carry out the Procedures according to the corresponding International Standards of Supreme Audit Institutions and code of ethics issued by INTOSAI instead of the International Standard on Related Services ('ISRS') 4400 and the Code of Ethics for Professional Accountants issued by the IAASB and the IESBA.

## **Independent report of factual findings on the methodology concerning grant agreements financed under the Horizon 2020 Research and Innovation Framework Programme**

*(To be printed on letterhead paper of the auditor)*

To

[ name of contact person(s)], [Position]  
[[Beneficiary's] [Linked Third Party's] name]  
[ Address]  
[ dd Month yyyy]

Dear [Name of contact person(s)],

As agreed under the terms of reference dated [dd Month yyyy]

with [OPTION 1: [insert name of the beneficiary] ('the Beneficiary')] [OPTION 2: [insert name of the linked third party] ('the Linked Third Party'), third party linked to the Beneficiary [insert name of the beneficiary] ('the Beneficiary')],

we

[ name of the auditor] ('the Auditor'),

established at

[full address/city/state/province/country],

represented by

[name and function of an authorised representative],

have carried out the agreed-upon procedures ('the Procedures') and provide hereby our Independent Report of Factual Findings ('the Report'), concerning the [Beneficiary's] [Linked Third Party's] usual accounting practices for calculating and declaring direct personnel costs declared as unit costs ('the Methodology').

You requested certain procedures to be carried out in connection with the grant(s)

[title and number of the grant agreement(s)] ('the Agreement(s)').

### **The Report**

Our engagement was carried out in accordance with the terms of reference ('the ToR') appended to this Report. The Report includes: the standard statements ('the Statements') made by the [Beneficiary] [Linked Third Party], the agreed-upon procedures ('the Procedures') carried out and the standard factual findings ('the Findings') confirmed by us.

The engagement involved carrying out the Procedures and assessing the Findings and the documentation requested appended to this Report, the results of which the Commission uses to draw conclusions regarding the acceptability of the Methodology applied by the [Beneficiary] [Linked Third Party].

The Report covers the methodology used from [dd Month yyyy]. In the event that the [Beneficiary] [Linked Third Party] changes this methodology, the Report will not be applicable to any Financial Statement<sup>1</sup> submitted thereafter.

The scope of the Procedures and the definition of the standard statements and findings were determined solely by the Commission. Therefore, the Auditor is not responsible for their suitability or pertinence.

Since the Procedures carried out constitute neither an audit nor a review made in accordance with International Standards on Auditing or International Standards on Review Engagements, we do not give a statement of assurance on the costs declared on the basis of the [Beneficiary's] [Linked Third Party's] Methodology. Had we carried out additional procedures or had we performed an audit or review in accordance with these standards, other matters might have come to its attention and would have been included in the Report.

### Exceptions

Apart from the exceptions listed below, the [Beneficiary] [Linked Third Party] agreed with the standard Statements and provided the Auditor all the documentation and accounting information needed by the Auditor to carry out the requested Procedures and corroborate the standard Findings.

**List here any exception and add any information on the cause and possible consequences of each exception, if known. If the exception is quantifiable, also indicate the corresponding amount.**

.....

*Explanation of possible exceptions in the form of examples (to be removed from the Report):*

- i. the [Beneficiary] [Linked Third Party] did not agree with the standard Statement number ... because...;
- ii. the Auditor could not carry out the procedure ... established because .... (e.g. due to the inability to reconcile key information or the unavailability or inconsistency of data);
- iii. the Auditor could not confirm or corroborate the standard Finding number ... because ....

### Remarks

We would like to add the following remarks relevant for the proper understanding of the Methodology applied by the [Beneficiary] [Linked Third Party] or the results reported:

*Example (to be removed from the Report):*

*Regarding the methodology applied to calculate hourly rates ...*

*Regarding standard Finding 15 it has to be noted that ...*

*The [Beneficiary] [Linked Third Party] explained the deviation from the benchmark statement XXIV concerning time recording for personnel with no exclusive dedication to the action in the following manner:*

...

### Annexes

Please provide the following documents to the auditor and annex them to the report when submitting this CoMUC to the Commission:

<sup>1</sup> Financial Statement in this context refers solely to Annex 4 of the Agreement by which the Beneficiary declares costs under the Agreement.

1. Brief description of the methodology for calculating personnel costs, productive hours and hourly rates;
2. Brief description of the time recording system in place;
3. An example of the time records used by the [Beneficiary] [Linked Third Party];
4. Description of any budgeted or estimated elements applied, together with an explanation as to why they are relevant for calculating the personnel costs and how they are based on objective and verifiable information;
5. A summary sheet with the hourly rate for direct personnel declared by the [Beneficiary] [Linked Third Party] and recalculated by the Auditor for each staff member included in the sample (the names do not need to be reported);
6. A comparative table summarising for each person selected in the sample a) the time claimed by the [Beneficiary] [Linked Third Party] in the Financial Statement(s) and b) the time according to the time record verified by the Auditor;
7. A copy of the letter of representation provided to the Auditor.

### Use of this Report

This Report has been drawn up solely for the purpose given under Point 1.1 Reasons for the engagement.

#### The Report:

- is confidential and is intended to be submitted to the Commission by the [Beneficiary] [Linked Third Party] in connection with Article 18.1.2 of the Agreement;
- may not be used by the [Beneficiary] [Linked Third Party] or by the Commission for any other purpose, nor distributed to any other parties;
- may be disclosed by the Commission only to authorised parties, in particular the European Anti-Fraud Office (OLAF) and the European Court of Auditors.
- relates only to the usual cost accounting practices specified above and does not constitute a report on the Financial Statements of the [Beneficiary] [Linked Third Party].

No conflict of interest<sup>2</sup> exists between the Auditor and the Beneficiary [and the Linked Third Party] that could have a bearing on the Report. The total fee paid to the Auditor for producing the Report was EUR [ ] (including EUR [ ] of deductible VAT).

We look forward to discussing our Report with you and would be pleased to provide any further information or assistance which may be required.

Yours sincerely

[legal name of the Auditor]

[name and title of the authorised representative]

[dd Month yyyy]

Signature of the Auditor

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<sup>2</sup> A conflict of interest arises when the Auditor's objectivity to establish the certificate is compromised in fact or in appearance when the Auditor for instance:

- was involved in the preparation of the Financial Statements;
- stands to benefit directly should the certificate be accepted;
- has a close relationship with any person representing the beneficiary;
- is a director, trustee or partner of the beneficiary; or
- is in any other situation that compromises his or her independence or ability to establish the certificate impartially.



**Statements to be made by the Beneficiary/Linked Third Party ('the Statements') and Procedures to be carried out by the Auditor ('the Procedures') and standard factual findings ('the Findings') to be confirmed by the Auditor**

The Commission reserves the right to provide the auditor with guidance regarding the Statements to be made, the Procedures to be carried out or the Findings to be ascertained and the way in which to present them. The Commission reserves the right to vary the Statements, Procedures or Findings by written notification to the Beneficiary/Linked Third Party to adapt the procedures to changes in the grant agreement(s) or to any other circumstances.

If this methodology certificate relates to the Linked Third Party's usual accounting practices for calculating and claiming direct personnel costs declared as unit costs any reference here below to 'the Beneficiary' is to be considered as a reference to 'the Linked Third Party'.

<i>Please explain any discrepancies in the body of the Report.</i>	
Statements to be made by Beneficiary	Procedures to be carried out and Findings to be confirmed by the Auditor
<p><b>A. Use of the Methodology</b></p> <p>I. The cost accounting practice described below has been in use since /dd Month yyyy/.</p> <p>II. The next planned alteration to the methodology used by the Beneficiary will be from [dd Month yyyy/.</p>	<p><b>Procedure:</b></p> <p>✓ The Auditor checked these dates against the documentation the Beneficiary has provided.</p> <p><b>Factual finding:</b></p> <p>1. The dates provided by the Beneficiary were consistent with the documentation.</p>
<p><b>B. Description of the Methodology</b></p> <p>III. The methodology to calculate unit costs is being used in a consistent manner and is reflected in the relevant procedures.</p> <p><i>[Please describe the methodology your entity uses to calculate <u>personnel costs</u>, productive hours and hourly rates, present your description to the Auditor and annex it to this certificate]</i></p> <p><i>[If the statement of section "B. Description of the methodology" cannot be endorsed by the Beneficiary or there is no written methodology to calculate unit costs it should be listed here below and reported as exception by the Auditor in the main Report of Factual Findings:</i></p> <p>- ...]</p>	<p><b>Procedure:</b></p> <p>✓ The Auditor reviewed the description, the relevant manuals and/or internal guidance documents describing the methodology.</p> <p><b>Factual finding:</b></p> <p>2. The brief description was consistent with the relevant manuals, internal guidance and/or other documentary evidence the Auditor has reviewed.</p> <p>3. The methodology was generally applied by the Beneficiary as part of its usual costs accounting practices.</p>

<i>Please explain any discrepancies in the body of the Report.</i>	
Statements to be made by Beneficiary	Procedures to be carried out and Findings to be confirmed by the Auditor
<p><b>C. Personnel costs</b></p> <p><u>General</u></p> <p>IV. The unit costs (hourly rates) are limited to salaries including during parental leave, social security contributions, taxes and other costs included in the remuneration required under national law and the employment contract or equivalent appointing act;</p> <p>V. Employees are hired directly by the Beneficiary in accordance with national law, and work under its sole supervision and responsibility;</p> <p>VI. The Beneficiary remunerates its employees in accordance with its usual practices. This means that personnel costs are charged in line with the Beneficiary's usual payroll policy (e.g. salary policy, overtime policy, variable pay) and no special conditions exist for employees assigned to tasks relating to the European Union or Euratom, unless explicitly provided for in the grant agreement(s);</p> <p>VII. The Beneficiary allocates its employees to the relevant group/category/cost centre for the purpose of the unit cost calculation in line with the usual cost accounting practice;</p> <p>VIII. Personnel costs are based on the payroll system and accounting system.</p> <p>IX. Any exceptional adjustments of actual personnel costs resulted from relevant budgeted or estimated elements and were based on objective and verifiable information. <i>[Please describe the 'budgeted or estimated elements' and their relevance to personnel costs, and explain how they were reasonable and based on objective and verifiable information, present your explanation to the Auditor and annex it to this certificate].</i></p> <p>X. Personnel costs claimed do not contain any of the following ineligible costs: costs related to return on capital; debt and debt service charges; provisions for future losses or debts; interest owed; doubtful debts; currency exchange losses; bank costs charged by the Beneficiary's bank for transfers from the Commission/Agency; excessive or reckless expenditure; deductible VAT or costs incurred during suspension of the implementation of the action.</p> <p>XI. Personnel costs were not declared under another EU or Euratom grant</p>	<p><b>Procedure:</b></p> <p><i>The Auditor draws a sample of employees to carry out the procedures indicated in this section C and the following sections D to F.</i>  <i>[The Auditor has drawn a random sample of 10 employees assigned to Horizon 2020 action(s). If fewer than 10 employees are assigned to the Horizon 2020 action(s), the Auditor has selected all employees assigned to the Horizon 2020 action(s) complemented by other employees irrespective of their assignments until he has reached 10 employees.].</i> For this sample:</p> <ul style="list-style-type: none"> <li>✓ the Auditor reviewed all documents relating to personnel costs such as employment contracts, payslips, payroll policy (e.g. salary policy, overtime policy, variable pay policy), accounting and payroll records, applicable national tax, labour and social security law and any other documents corroborating the personnel costs claimed;</li> <li>✓ in particular, the Auditor reviewed the employment contracts of the employees in the sample to verify that:             <ul style="list-style-type: none"> <li>i. they were employed directly by the Beneficiary in accordance with applicable national legislation;</li> <li>ii. they were working under the sole technical supervision and responsibility of the latter;</li> <li>iii. they were remunerated in accordance with the Beneficiary's usual practices;</li> <li>iv. they were allocated to the correct group/category/cost centre for the purposes of calculating the unit cost in line with the Beneficiary's usual cost accounting practices;</li> </ul> </li> <li>✓ the Auditor verified that any ineligible items or any costs claimed under other costs categories or costs covered by other types of grant or by other grants financed from the European Union budget have not been taken into account when calculating the personnel costs;</li> <li>✓ the Auditor numerically reconciled the total amount of personnel costs used to calculate the unit cost with the total amount of personnel costs recorded in the statutory accounts and the payroll system.</li> </ul>

<i>Please explain any discrepancies in the body of the Report.</i>	
Statements to be made by Beneficiary	Procedures to be carried out and Findings to be confirmed by the Auditor
<p>(including grants awarded by a Member State and financed by the EU budget and grants awarded by bodies other than the Commission/Agency for the purpose of implementing the EU or Euratom budget in the same period, unless the Beneficiary can demonstrate that the operating grant does not cover any costs of the action).</p> <p><u>If additional remuneration as referred to in the grant agreement(s) is paid</u></p> <p>XII. The Beneficiary is a non-profit legal entity;</p> <p>XIII. The additional remuneration is part of the beneficiary's usual remuneration practices and paid consistently whenever the relevant work or expertise is required;</p> <p>XIV. The criteria used to calculate the additional remuneration are objective and generally applied regardless of the source of funding;</p> <p>XV. The additional remuneration included in the personnel costs used to calculate the hourly rates for the grant agreement(s) is capped at EUR 8 000 per full-time equivalent (reduced proportionately if the employee is not assigned exclusively to the action).</p> <p><i>[If certain statement(s) of section "C. Personnel costs" cannot be endorsed by the Beneficiary they should be listed here below and reported as exception by the Auditor in the main Report of Factual Findings:</i></p> <p>- ...]</p>	<ul style="list-style-type: none"> <li>✓ to the extent that actual personnel costs were adjusted on the basis of budgeted or estimated elements, the Auditor carefully examined those elements and checked the information source to confirm that they correspond to objective and verifiable information;</li> <li>✓ if additional remuneration has been claimed, the Auditor verified that the Beneficiary was a non-profit legal entity, that the amount was capped at EUR 8 000 per full-time equivalent and that it was reduced proportionately for employees not assigned exclusively to the action(s).</li> <li>✓ the Auditor recalculated the personnel costs for the employees in the sample.</li> </ul> <p><b>Factual finding:</b></p> <ol style="list-style-type: none"> <li>4. All the components of the remuneration that have been claimed as personnel costs are supported by underlying documentation.</li> <li>5. The employees in the sample were employed directly by the Beneficiary in accordance with applicable national law and were working under its sole supervision and responsibility.</li> <li>6. Their employment contracts were in line with the Beneficiary's usual policy;</li> <li>7. Personnel costs were duly documented and consisted solely of salaries, social security contributions (pension contributions, health insurance, unemployment fund contributions, etc.), taxes and other statutory costs included in the remuneration (holiday pay, thirteenth month's pay, etc.);</li> <li>8. The totals used to calculate the personnel unit costs are consistent with those registered in the payroll and accounting records;</li> <li>9. To the extent that actual personnel costs were adjusted on the basis of budgeted or estimated elements, those elements were relevant for calculating the personnel costs and correspond to objective and verifiable information. The budgeted or estimated elements used are: — (indicate the elements and their values).</li> <li>10. Personnel costs contained no ineligible elements;</li> <li>11. Specific conditions for eligibility were fulfilled when additional</li> </ol>

<i>Please explain any discrepancies in the body of the Report.</i>	
Statements to be made by Beneficiary	Procedures to be carried out and Findings to be confirmed by the Auditor
	remuneration was paid: a) the Beneficiary is registered in the grant agreements as a non-profit legal entity; b) it was paid according to objective criteria generally applied regardless of the source of funding used and c) remuneration was capped at EUR 8000 per full-time equivalent (or up to up to the equivalent pro-rata amount if the person did not work on the action full-time during the year or did not work exclusively on the action).
<p><b>D. Productive hours</b></p> <p>XVI. The number of productive hours per full-time employee applied is <i>[delete as appropriate]</i>:</p> <p>A. 1720 productive hours per year for a person working full-time (corresponding pro-rata for persons not working full time).</p> <p>B. the total number of hours worked in the year by a person for the Beneficiary</p> <p>C. the standard number of annual hours generally applied by the beneficiary for its personnel in accordance with its usual cost accounting practices. This number must be at least 90% of the standard annual workable hours.</p> <p><u>If method B is applied</u></p> <p>XVII. The calculation of the total number of hours worked was done as follows: annual workable hours of the person according to the employment contract, applicable labour agreement or national law plus overtime worked minus absences (such as sick leave and special leave).</p> <p>XVIII. ‘Annual workable hours’ are hours during which the personnel must be working, at the employer’s disposal and carrying out his/her activity or duties under the employment contract, applicable collective labour agreement or national working time legislation.</p> <p>XIX. The contract (applicable collective labour agreement or national working time legislation) do specify the working time enabling to calculate the annual workable hours.</p>	<p><b>Procedure (same sample basis as for Section C: Personnel costs):</b></p> <ul style="list-style-type: none"> <li>✓ The Auditor verified that the number of productive hours applied is in accordance with method A, B or C.</li> <li>✓ The Auditor checked that the number of productive hours per full-time employee is correct.</li> <li>✓ If method B is applied the Auditor verified i) the manner in which the total number of hours worked was done and ii) that the contract specified the annual workable hours by inspecting all the relevant documents, national legislation, labour agreements and contracts.</li> <li>✓ If method C is applied the Auditor reviewed the manner in which the standard number of working hours per year has been calculated by inspecting all the relevant documents, national legislation, labour agreements and contracts and verified that the number of productive hours per year used for these calculations was at least 90% of the standard number of working hours per year.</li> </ul> <p><b>Factual finding:</b></p> <p><u>General</u></p> <p>12. The Beneficiary applied a number of productive hours consistent with method A, B or C detailed in the left-hand column.</p> <p>13. The number of productive hours per year per full-time employee was accurate.</p> <p><u>If method B is applied</u></p> <p>14. The number of ‘annual workable hours’, overtime and absences was</p>

<i>Please explain any discrepancies in the body of the Report.</i>	
Statements to be made by Beneficiary	Procedures to be carried out and Findings to be confirmed by the Auditor
<p><u>If method C is applied</u></p> <p>XX. The standard number of productive hours per year is that of a full-time equivalent.</p> <p>XXI. The number of productive hours per year on which the hourly rate is based i) corresponds to the Beneficiary's usual accounting practices; ii) is at least 90 % of the standard number of workable (working) hours per year.</p> <p>XXII. Standard workable (working) hours are hours during which personnel are at the Beneficiary's disposal performing the duties described in the relevant employment contract, collective labour agreement or national labour legislation. The number of standard annual workable (working) hours that the Beneficiary claims is supported by labour contracts, national legislation and other documentary evidence.</p> <p><i>[If certain statement(s) of section "D. Productive hours" cannot be endorsed by the Beneficiary they should be listed here below and reported as exception by the Auditor:]</i>  - ...]</p>	<p>verifiable based on the documents provided by the Beneficiary and the calculation of the total number of hours worked was accurate.</p> <p>15. The contract specified the working time enabling to calculate the annual workable hours.</p> <p><u>If method C is applied</u></p> <p>16. The calculation of the number of productive hours per year corresponded to the usual costs accounting practice of the Beneficiary.</p> <p>17. The calculation of the standard number of workable (working) hours per year was corroborated by the documents presented by the Beneficiary.</p> <p>18. The number of productive hours per year used for the calculation of the hourly rate was at least 90 % of the number of workable (working) hours per year.</p>
<p><b>E. Hourly rates</b></p> <p>The hourly rates are correct because:</p> <p>XXIII. Hourly rates are correctly calculated since they result from dividing annual personnel costs by the productive hours of a given year and group (e.g. staff category or department or cost centre depending on the methodology applied) and they are in line with the statements made in section C. and D. above.</p> <p><i>[If the statement of section 'E. Hourly rates' cannot be endorsed by the Beneficiary they should be listed here below and reported as exception by the Auditor:]</i>  - ...]</p>	<p><b>Procedure</b></p> <ul style="list-style-type: none"> <li>✓ The Auditor has obtained a list of all personnel rates calculated by the Beneficiary in accordance with the methodology used.</li> <li>✓ The Auditor has obtained a list of all the relevant employees, based on which the personnel rate(s) are calculated.</li> </ul> <p>For 10 employees selected at random (same sample basis as Section C: Personnel costs):</p> <ul style="list-style-type: none"> <li>✓ The Auditor recalculated the hourly rates.</li> <li>✓ The Auditor verified that the methodology applied corresponds to the usual accounting practices of the organisation and is applied consistently for all activities of the organisation on the basis of objective criteria irrespective of the source of funding.</li> </ul> <p><b>Factual finding:</b></p>

<i>Please explain any discrepancies in the body of the Report.</i>	
Statements to be made by Beneficiary	Procedures to be carried out and Findings to be confirmed by the Auditor
	19. No differences arose from the recalculation of the hourly rate for the employees included in the sample.
<p><b>F. Time recording</b></p> <p>XXIV. Time recording is in place for all persons with no exclusive dedication to one Horizon 2020 action. At least all hours worked in connection with the grant agreement(s) are registered on a <b>daily/weekly/monthly</b> basis <i>[delete as appropriate]</i> using a <b>paper/computer-based system</b> <i>[delete as appropriate]</i>;</p> <p>XXV. For persons exclusively assigned to one Horizon 2020 activity the Beneficiary has either signed a declaration to that effect or has put arrangements in place to record their working time;</p> <p>XXVI. Records of time worked have been signed by the person concerned (on paper or electronically) and approved by the action manager or line manager at least monthly;</p> <p>XXVII. Measures are in place to prevent staff from:</p> <ol style="list-style-type: none"> <li>recording the same hours twice,</li> <li>recording working hours during absence periods (e.g. holidays, sick leave),</li> <li>recording more than the number of productive hours per year used to calculate the hourly rates, and</li> <li>recording hours worked outside the action period.</li> </ol> <p>XXVIII. No working time was recorded outside the action period;</p> <p>XXIX. No more hours were claimed than the productive hours used to calculate the hourly personnel rates.</p> <p><i>[Please provide a brief description of the <u>time recording system</u> in place together with the measures applied to ensure its reliability to the Auditor and annex it to the</i></p>	<p><b>Procedure</b></p> <ul style="list-style-type: none"> <li>✓ The Auditor reviewed the brief description, all relevant manuals and/or internal guidance describing the methodology used to record time.</li> </ul> <p>The Auditor reviewed the time records of the random sample of 10 employees referred to under Section C: Personnel costs, and verified in particular:</p> <ul style="list-style-type: none"> <li>✓ that time records were available for all persons with not exclusive assignment to the action;</li> <li>✓ that time records were available for persons working exclusively for a Horizon 2020 action, or, alternatively, that a declaration signed by the Beneficiary was available for them certifying that they were working exclusively for a Horizon 2020 action;</li> <li>✓ that time records were signed and approved in due time and that all minimum requirements were fulfilled;</li> <li>✓ that the persons worked for the action in the periods claimed;</li> <li>✓ that no more hours were claimed than the productive hours used to calculate the hourly personnel rates;</li> <li>✓ that internal controls were in place to prevent that time is recorded twice, during absences for holidays or sick leave; that more hours are claimed per person per year for Horizon 2020 actions than the number of productive hours per year used to calculate the hourly rates; that working time is recorded outside the action period;</li> <li>✓ the Auditor cross-checked the information with human-resources records to verify consistency and to ensure that the internal controls have been effective. In addition, the Auditor has verified that no more hours were charged to Horizon 2020 actions per person per year than the number of productive hours per year used to calculate the hourly rates, and verified that</li> </ul>



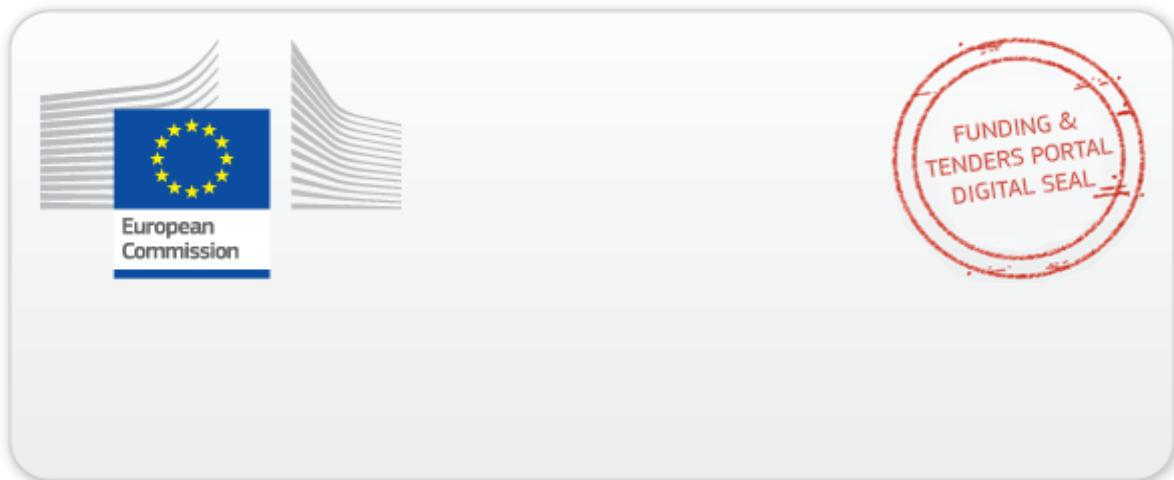
<i>Please explain any discrepancies in the body of the Report.</i>	
Statements to be made by Beneficiary	Procedures to be carried out and Findings to be confirmed by the Auditor
<p><i>present certificate<sup>1</sup>].</i></p> <p><i>[If certain statement(s) of section “F. Time recording” cannot be endorsed by the Beneficiary they should be listed here below and reported as exception by the Auditor: - ...]</i></p>	<p>no time worked outside the action period was charged to the action.</p> <p><b>Factual finding:</b></p> <ol style="list-style-type: none"> <li>20. The brief description, manuals and/or internal guidance on time recording provided by the Beneficiary were consistent with management reports/records and other documents reviewed and were generally applied by the Beneficiary to produce the financial statements.</li> <li>21. For the random sample time was recorded or, in the case of employees working exclusively for the action, either a signed declaration or time records were available;</li> <li>22. For the random sample the time records were signed by the employee and the action manager/line manager, at least monthly.</li> <li>23. Working time claimed for the action occurred in the periods claimed;</li> <li>24. No more hours were claimed than the number productive hours used to calculate the hourly personnel rates;</li> <li>25. There is proof that the Beneficiary has checked that working time has not been claimed twice, that it is consistent with absence records and the number of productive hours per year, and that no working time has been claimed outside the action period.</li> <li>26. Working time claimed is consistent with that on record at the human-resources department.</li> </ol>

<sup>1</sup> The description of the time recording system must state among others information on the content of the time records, its coverage (full or action time-recording, for all personnel or only for personnel involved in H2020 actions), its degree of detail (whether there is a reference to the particular tasks accomplished), its form, periodicity of the time registration and authorisation (paper or a computer-based system; on a daily, weekly or monthly basis; signed and countersigned by whom), controls applied to prevent double-charging of time or ensure consistency with HR-records such as absences and travels as well as its information flow up to its use for the preparation of the Financial Statements.

Grant Agreement number: [insert number] [insert acronym] [insert call identifier]

H2020 Model Grant Agreements: H2020 General MGA — Multi: v5.0 – dd.mm.2017

<i>Please explain any discrepancies in the body of the Report.</i>	
Statements to be made by Beneficiary	Procedures to be carried out and Findings to be confirmed by the Auditor
<i>[official name of the [Beneficiary] [Linked Third Party]]</i>	<i>[official name of the Auditor]</i>
<i>[name and title of authorised representative]</i>	<i>[name and title of authorised representative]</i>
<i>[dd Month yyyy]</i>	<i>[dd Month yyyy]</i>
<i>&lt;Signature of the [Beneficiary] [Linked Third Party]&gt;</i>	<i>&lt;Signature of the Auditor&gt;</i>



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